

nature

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Filling Bondi's place

PROFESSOR Sir Hermann Bondi, profiled this week (page 201), took up his post as chief scientist at the UK Department of Energy on 1 October. That the post he left at the Ministry of Defence has remained unfilled for a comparatively short while should not diminish concern over the way this and similar appointments are made.

Bondi's former post is the highest in government which any scientist can hope to achieve. Its holder has the stature to receive the most privileged information, and the access to offer independent advice, across the broadest range of issues, at the highest levels of government. To have it vacant longer than necessary is thus undesirable. Yet the Ministry of Defence has known of Bondi's departure for nearly five months—possibly longer.

The delay has a simple and not unreasonable explanation. Because the post is also the highest which can be occupied by someone from outside both political and civil service ranks, the appointee must be acceptable to those he will work with—service chiefs as well as ministers and civil servants. The choice will be further limited by the need for

a 'link man' with the scientific community who has standing and prestige and who is also willing to give up his job. And even when the right man is found, there remains the crucial matter of security clearance.

The question is whether such an important appointment, of such consequence for the public interest, should be made more openly even though—perhaps because—the number of candidates is so restricted. The argument against this is that to name names would subject potential candidates to considerable pressures in their existing jobs, and that the consequences of a failed security clearance would be fearfully embarrassing—all without much good being done. But this does not mean that public canvassing of names cannot work. Indeed, it works elsewhere, notably in the United States, where experience with it is admittedly far greater. The public interest aspect aside, the US system may even work better. If so, the question now is whether a change could be implemented smoothly in Britain without ending up with the worst of both systems. The trouble is, no one is involved in or even encouraging discussion of it. □

New frontiers at Trieste

THE International Centre for Theoretical Physics (ICTP) at Trieste, dedicated to the support of physicists in the developing world, lives on several knife-edges. For one thing it relies on comparable contributions for its support from the international agencies UNESCO and IAEA and also on the continued financial goodwill of the Italian government and the City of Trieste. For another there are many, scientists, included, who wonder whether theoretical physics is a luxury that it is no business of the developed world to stimulate by means of courses at a very high level. Yet again, inevitably the quality of those who attend is much more variable than, say, amongst a group of students with a common background; thus the Centre will have more than its fair share of visitors who feel lost in its environment. And finally, there are certainly those who say 'if theoretical physics, why not experimental biology', and so on.

The Centre, under the guidance of Abdus Salam, has not, however, put up the shutters against criticism. Rather, it has been making moves to add new disciplines to its range of interests. Thus in the past couple of years it has held substantial courses on the teaching of physics at French-speaking universities in the developing world, on solar-energy conversion, on the physics of oceans and the atmosphere and, most recently, on the physics of the Earth. There is talk, also, of the possibility of a new experimental centre to be built on the Yugoslav/Italian border.

As the centre adds new peripheral interests to its core subjects of high-energy and nuclear physics, solid state physics and applicable mathematics, it finds itself closer to the tricky problems of science and society. Indeed the oceans and atmosphere course was stimulated by the Bangladesh floods, and the physics of the Earth course by the Friuli earthquake of last year which shook Trieste. Out of such courses can come politically relevant action, as happened last week. Students on the course were presented with lectures on earthquake-prediction research as practised in the United States, Soviet Union, Italy, China and

Japan. But the question arose, what should developing countries be doing about this? Many of the worst losses of life and property occur in the developing world; are such countries to wait supinely until systems are perfected elsewhere, and then be confronted with a very expensive package? Yet if not, most developing countries could not mount a major research initiative.

The need, then, is for a middle way which encourages scientists to keep in touch with developments and accumulate the necessary background information and which convinces politicians that there are human and economic benefits to treating the matter seriously. The centre, it emerged during discussions, could play a significant role in assisting technology transfer. The proposal was that ICTP, with its devotion to scientific excellence and its easy relations with scientists from a wide range of developing countries, should take a formal and special interest in ensuring that such scientists are kept fully in touch with developments in prediction and the assessment of seismic risk. ICTP already takes a similar special interest in solid state physics, a subject with relevance to the developing world.

This initiative should be widely welcomed. (The Editor declares an interest, having been involved in the discussions.) Of course there are international unions and associations which could also have performed this function to a degree, but satisfaction at the possible commitment of ICTP in this work will far outweigh any quibbles over who else could have done what.

In the longer run ICTP, in conjunction with UNESCO maybe, could possibly participate more actively in the raising of political consciousness on earthquake hazards. A model already exists in the team of examiners which occasionally emerges from OECD in Paris to visit a developed country and examine its science policy. A team of experts in earthquake protection could perform a similar role of persuading politicians to take their duties in hazard reduction seriously. □



The North American North (1)

What strategy for development?

David Spurgeon in Ottawa summarises a Science Council report on the development of Canada's North

LITTLE-KNOWN to the rest of the world, Canada is a country about which myth and misconception abound. Many people are especially ignorant of Canada's North, but that is scarcely to be wondered at, for so are Canadians themselves. And lately, many of the myths have been challenged from within, including the myth of self-sufficiency in energy and minerals. No less than the economic adviser to the Progressive Conservative party, James Gillies, has wondered about Canada as a technologically advanced nation and suggested that the country should return to reliance on selling its natural resources.

This orgy of self examination has also included some fundamental studies of the North, and the latest is a report from the Science Council of Canada. Called *Northward Looking: A Strategy and a Science Policy for Northern Development*, it is the result of 3½ years' preparation and sets out a strategy designed to bring more economic and technological self-sufficiency to the North, and a set of principles that should guide science policies for northern development.

But to set the stage, it tells Canadians a few facts about the part of their country—the largest part—that few of them have ever seen. The perspective bears repetition. Canada is the second largest country in the world, and the Yukon and the Northwest Territories together comprise 41% of its land area. As the report puts it:

Canada ranks 33rd among countries of the world in population. There is no settlement in 89% of our land area. In 1971, our average population density south of 60 degrees was 1,024 people per hundred square miles; for Nova Scotia, 3,867; for Ontario, 2,239. In the Yukon it was 9, and in the Northwest Territories, 3. Half of the people in the Northwest Territories and 60% of those in the Yukon live in *urban* environments. The northern part of Canada is mostly a lonesome place.

The North, says the report, is characterised by "small populations, a short growing season, permafrost, and long, cold, dark winters. The Extreme North and the Middle North are very different. The Extreme North is nearly uninhabited, has very little vegetation, continuous permafrost, ice-infested waters all or nearly all year, and very little precipitation. The Middle North has discontinuous permafrost, is heavily forested, more accessible, and is the

focus of resource exploration at this time. It is the region where the large majority of northern residents now live".

The people of the North, as distinctive as their environment, present problems for demographers. They move over large areas, there are cultural and linguistic differences, and many settlements are relatively isolated. Demographic statistics are thus more crude than elsewhere in the country. No accurate population figures exist on Métis and non-status Indians in Canada. The Native Council of Canada estimates 750,000 people on the basis of three Métis for each status Indian (295,215 in 1971 in all Canada). The total population of the North is about one million. Compared with the rest of Canada, they are younger and have received less formal education. There is a larger component of native people (especially in Saskatchewan, Manitoba, Quebec, and the Northwest Territories), and larger proportions of the population are engaged in forestry, fishing, trapping, and mining than in Canada as a whole.

Large communities are uncommon in the North: in 1971 only 29% lived in settlements of over 10,000, compared to 65% in Canada as a whole. Within the North, a community of more than 2,000 is an urban environment. Most of the immigrants settle in the larger centres: more than 70% of the non-natives live in the five largest communities of the Northwest Territories—Yellowknife, Inuvik, Hay River, Fort Smith and Frobisher Bay.

Epidemiological data indicate that health standards in the North are lower than in the rest of Canada. And although the birth rate has now begun to level off, the age structure of the native population resembles that of Latin America or Africa more than the rest of Canada. Twenty per cent of the inhabitants of the Northwest Territories speak neither English nor French, there are several native dialects, and in the Mackenzie River Valley and Delta, six native languages. "There is not really just one North in Canada", says the report. "The diversity of the North is as striking as its cold".

Historical overview first

To study this area, the council undertook a historical overview and case

studies of northern development. The work was synthesised in a discussion paper, which then served as the basic document for seminars and to solicit opinion from more than 100 people involved in northern affairs. All this led to the framing of the final report. The chairman of the Science Council Committee on Northern Development, W. H. Gauvin, notes in an introduction that the process covered a period when many important issues were emerging: the OPEC oil embargo, land claims by natives in the James Bay area, the first drilling from artificial islands in the Beaufort Sea, preparations for northern pipelines and extraction of oil from the sands and so on.

It is against this background that the report's recommendations must be set. It notes that the recent history of the North has been the product of two conflicting trends: the thrust toward large-scale exploitation of natural resources, and the desire to continue traditional resource harvesting activities such as fishing, hunting and trapping, which would lead to development based on smaller scale, locally controlled projects.

"The Science Council believes that both major trends should be accommodated in a *strategy of mixed development*", says the report. "Such a strategy would press for more economic and technological self-sufficiency for the North. Activities that can be logically defined and controlled would be favoured over those which tend to increase political and economic dependence, the need for welfare, or other undesirable social conditions. This means an emphasis on relatively low capital, decentralised, and small scale development".

The objectives of northern development espoused by the Science Council are similar to those expressed by the federal government: to promote the welfare of northern people, to maintain and enhance the regenerative capacity of the environment, to give renewable resource development a higher priority, and to encourage economically viable non-renewable resource projects that are in the national interest and will benefit—or at least not harm—northern residents and their environment.

This strategy of mixed development will require greater sensitivity to traditional patterns of land use, says the report. It also says it will involve extracting mineral resources more slowly than some "major participants" may desire. Ironically, shortly after the report appeared, the Energy Minister, Alastair Gillespie, was proposing encouraging exploration by the multinational oil companies by offering them tax cuts. The report emphasises

that the land has special significance for many native northerners, and needs to be valued in terms of its capability to meet a variety of needs not measurable in monetary terms.

The report also calls for greater recognition of the value of public participation in projects concerning the North. "The right of people directly affected by a project to have something to say about it is becoming more widely accepted. . . . In the North, in fact, complaints about adequate *consultation* have been so frequent that the question of *participation* itself is only now being raised. To pursue successfully the strategy of mixed development, there must be more than informing, educating, and consulting northern people about the needs of the rest of Canada for a certain project".

The report lays down four principles that should guide policies for northern development:

- Technological sovereignty: the ability of Canadians to control, direct and benefit from technological enterprises deemed essential to the country.
- Life-style flexibility: the need to allow opportunities for choices of life style.
- Maintenance of the regenerative capacity of the land.
- Comprehensive and balanced assessment and monitoring of large and small projects.

These four principles, it says, should govern the choice of all new research and development initiatives in the North.

Technological sovereignty has become a buzz word at the Science Council. The reason for applying it to the North is that there, as elsewhere, "Canadians and Canadian science and industry tend to place undue emphasis on foreign expertise and foreign consultants". The report says that foreign-owned firms tend to perform the research that has the greatest potential for long-term payoffs in their home countries.

In offshore Labrador, for example, where several companies are searching for oil and developing the expertise necessary for that environment, Canadian firms have participated in the data collection phase, but they have had very little to do with planning and design of production and transportation facilities. The nature of research activity in Canadian resource-related industries, then, tends to resemble the resource extraction industries themselves. That is, with few exceptions, Canadians provide the raw materials, but the control of the operation, the processing and, hence, long-term benefits tend to flow out of the country.

In order to support the proposed strategies for northern development the Science Council makes a number of proposals. One is that universities should play a greater role in the solu-

tion of northern problems. In spite of the efforts of the universities of Alberta, Saskatchewan, McGill, Chicoutimi, Laval, Memorial (in Newfoundland) and others, most northern research is now performed by industry and government. Canada needs a cadre of researchers not dependent on contracts from interested parties, the report says. The Science Council urges that funds for northern research be re-allocated so that grants are emphasised over contracts, and that the granting councils provide funds for logistic support over and above other costs, because northern research is costly.

In addition, the Council urges the establishment of a University of the North to provide the focus for northern research. This university should concentrate on such areas as resource management and systematising resource inventories, and should promote the innovation of northern technologies. Native peoples should play a central role in the choice of

research topics and in undertaking research. Funding should be primarily from the federal government, and the university at first would give only graduate degrees.

The report emphasises the need for an inventory of renewable resources, and other data on the North, and the need for improved communications. There is also a need for coordination and exchange of the information that would come out of a northern data bank system, particularly among those who fund northern research. "It is intolerable that Members of Parliament should regularly report difficulties in gaining access to technical and scientific information relevant to political decision-making", it says.

In the past, most scientific exploration of Canada's North has been carried out by scientists of other countries, and much of the North's development has been for the benefit of others. If the Science Council's recommendations are acted upon, that era may end. □

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Camera Press

Bleak outpost at Hudson's Bay

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National Film Board

Prospecting for metals in Canada's North

The North American North (2)

Money or energy problem?

Jeff Carruthers in Ottawa examines the most expensive private capital project ever undertaken, the AlCan gas pipeline

CANADA and the United States have together decided to build an all-new gas transmission system to carry Alaskan natural gas more than 5,000 miles to markets in North America's industrial heartlands in the North and West. The planned pipeline can be described in many ways, all of them large. The cost alone will be at least \$10,000 million. Ultimately the system might also transport smaller gas reserves from Canada's nearby petroleum fields in the Mackenzie Delta and Beaufort Sea region of the western Canadian Arctic, to its own consumers in central and eastern Canada.

From a financial perspective, the Alaska Highway pipeline project is almost certainly the most expensive private capital project ever undertaken. Economists and bankers generally agree that financing could stretch Canadian and American money markets to their limit, especially if the inflation and cost over-runs that have plagued other recent North American energy projects (such as the trans-Alaska oil pipeline and the mammoth James Bay hydroelectric project) push costs for the Alaska Highway project to the \$15,000 million or \$20,000 million that some experts fear.

From a technical viewpoint, the problems to be encountered in the northernmost areas in Alaska and the Canadian Yukon Territory are considerable, but at the same time not regarded as insoluble. The key issue is whether they can be resolved within the time and cost parameters agreed to by the two governments. A frigid and dark climate prevails during the winter construction months. Gravel and water are difficult to find in places for construction. The permafrost, where materials hard as rock in below-zero temperatures can turn to soup if thawed, and where a phenomenon called 'frost heave' can literally lift a pipeline out of the ground if it is intentionally chilled through water-rich permafrost soils, presents its own problems. And an ecology that in many places is poorly understood needs protecting.

Economically speaking, Canada regards the pipeline project almost as a godsend, calculating that it could provide upwards of 100,000 man-years of direct and indirect employment for an economy facing rising unemployment, a worsening balance of trade and a damaging rate of inflation. In fact the

project is widely regarded as much as a make-work scheme as a vital (if costly) way of developing urgently-needed energy supplies.

Energy initiative

Yet it is the energy initiative inherent in the pipeline project which puts the Alaska Highway cooperative venture into its proper perspective. For \$10,000 million the United States hopes to connect gas reserves of 22–24 trillion cubic feet (TCF) in Alaska's Prudhoe Bay oil fields to markets in the lower 48 states. Assuming all of that gas is available—and there is some controversy about this already, based on fears that extraction of the gas could lower the recovery of associated (and more vital) crude oil—it would be sufficient to fill one year of current US gas demand.

Canada, which is hoping the pipeline can be connected later to some 5.2 TCF of gas in the Mackenzie Delta, would be able to fill two years of its current demand with its northern gas from the Delta. Ironically, perhaps, Canada is slated to export more natural gas from already-connected fields in southern Canada to the United States during the next decade than it hopes to connect in the western Arctic—and this is gas costing considerably less than Alaskan and Delta gas (the best estimates at present are that the Alaska Highway pipeline will deliver northern gas to southern markets for between \$3 and \$4 a thousand cubic feet, assuming no major cost over-runs for the pipeline project).

Critics of the pipeline include church groups, energy conservation groups, and environmentalists and northern native groups. They have argued that Canada and the United States do not really need the extra gas and that the adoption of adequate energy conservation techniques nationally could probably do more good for the state of the two nations' energy than any mammoth project. They have managed to focus national attention on a stark reality: that replacement energy for wasted existing energy resources in Canada is costing so much to develop on a per unit basis that even supposedly resource-rich countries like Canada cannot continue to rely on the non-renewable fossil fuels indefinitely.

If anything, the Alaska Highway pipeline underlines the growing belief that the availability of money is rapidly becoming more critical than the avail-



British Petroleum

Building the trans-Alaska oil pipeline

ability of new energy resources, especially as the new resources grow more and more remote either geographically, as with Arctic gas and oil, or technologically, as with Canada's vast oil sands deposits.

Two competitors

This relatively new concern about finance, which ultimately translates into energy costs to consumers, was one of the key factors in the two governments' selection of the Alaska Highway project over two competing gas transmission projects.

One rival consortium had proposed a pipeline from the oil and gas fields across the environmentally-sensitive North Slope (with the Alaska Wildlife range) to the Mackenzie River Delta, then up the Mackenzie River valley in the Northwest Territories to the province of Alberta, and then into the United States and central Canada. This proposal, by Canadian Arctic Gas Pipelines Ltd, a US-dominated consortium, was rejected in Canada on socio-economic and environmental grounds, even though it was believed to be technically superior to the Alaska Highway project. Approval of the project would have meant opening up the relatively lush Mackenzie Valley and would have risked confrontation with militant native groups, which are still trying to settle aboriginal claims with the Canadian government for much of the area.

The Arctic Gas project, as it was called, was the front-runner until a few months before the final government decision. A historic northern inquiry by a provincial Supreme Court judge, Thomas Berger, had been launched by the government to study the impact of such a development on the North generally and on the Mackenzie Valley specifically. Berger's report dealt a death blow to the Arctic Gas project when it suggested that the native way

of life for northern Eskimos and Indians would be ruined should the pipeline proceed within the decade. Berger looked more favourably on the Alaska Highway project, in part because a highway to Alaska had already disrupted that part of the North and because the natives were already more integrated into a southern style of life.

Other factors helped to kill the Arctic Gas project. The small Canadian gas reserves in the western Arctic, for example, did not justify an immediate Canadian commitment to a joint pipeline with the United States (the Alaska Highway project, by contrast is intended initially to serve only the United States). Arctic Gas also said it needed government guarantees to be able to finance its slightly-larger project—guarantees neither government was willing to provide. In addition, 'frost heave' and winter construction problems were more severe along the Mackenzie Valley route (the consortium would have had to provide 24-hour-a-day artificial lighting and artificial snow to construct the most northerly portions). Finally, the Arctic Gas project probably wouldn't have been Canadian controlled.

In the last analysis, the Arctic Gas project was an engineering and technical solution, based also on geological prospects along the route. But it failed to be sufficiently flexible to cope with non-technical matters, including its impact on native northerners, on Caribou herds, and on the ecology of a major river valley and a delta in the Arctic—not to mention political matters such as Canadian nationalism. To add further to the irony, the Arctic Gas proposal was studied to a much greater extent than the Alaska Highway project, so that more of the problems associated with it were known and widely publicised by its opponents in the media and before regulatory

review bodies.

Another petroleum industry consortium, preparing officially to propose the construction of an even longer and more technically difficult gas pipeline from the High Arctic of Canada (Melville Island initially) to southern markets, is keenly aware of this danger of being too prepared. The Polar Gas consortium, as it is called, is proposing a step-by-step government review in which the broad economic, social and environmental issues are dealt with first, preferably to produce some sort of initial government approval. Then more studies would be done and more money spent on the technical aspects of getting a 42-inch gas pipeline safely to the mainland, across marine trenches tens of miles wide and as much as 1,500 feet deep. The Polar Gas project is expected to cost even more than the Alaska Highway project, and to tap 10 to 15 TCF of gas for Canadian and possibly American markets.

Second project

The other project defeated by the Alaska Highway pipeline was the El Paso scheme to transport the Alaskan gas in liquified form by tankers to the west coast. The US government decided that a pipeline across Canada was more economical and involved less risk because a well-proven technology was being used. Since that decision earlier this year a number of Canadian companies (including PetroCanada, the national petroleum company, and Alberta Gas Trunk Line Company Ltd of Calgary, one of the two founders of the Alaska Highway pipeline consortium in Canada) have been studying the feasibility of using special ice-strengthened liquified natural gas tankers to transport High Arctic Islands gas from Melville Island to markets along the eastern seaboard. The project, if it is ever pursued, will initially move only 250,000 cubic feet a day, compared to more than 2,000 million cubic feet a day to be moved along the Alaska Highway pipeline starting in 1983.

In allowing the Alaska Highway pipeline to cross Canada, the Canadian government has insisted that the Canadian portions be Canadian controlled and has accepted the promises by private industry (the Foothills Pipelines Ltd consortium) that Canadian content in the Canadian portions would exceed 90% for goods and services. The government is assuming that in a few years the go-ahead will also be given to a lateral pipeline to the Mackenzie Delta, and space has been reserved in the pipeline for this Canadian gas.

The US government has even agreed to have its gas consumers pay for a

substantial portion of the lateral pipeline to the Mackenzie Delta, on the condition that cost over-runs in the Canadian sections of the main pipeline are kept below 35%. As a further incentive to keep costs under control, and with the nightmare cost over-runs associated with the TransAlaska oil pipeline still fresh in mind, the two governments plan to tie approved return on equity for the private participants to the success in minimising costs.

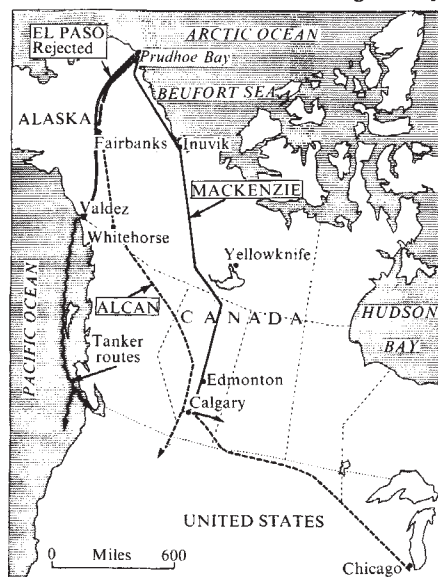
The US Congress has recently given legislative approval for the project. Legislation should be introduced in the Canadian parliament before the end of the year which will also establish a single monitoring agency to ensure that environmental and native impact is minimised and to ensure that safety standards are met.

Finance is the next major hurdle, with \$8,100 million due to be raised in US capital markets and \$1,700 million in Canada. The first official attempts at financing should take place late next year. And should private financing in North America fail, the pipeline consortium is expected to seek government assistance in Washington and Ottawa before trying to tap European and other foreign money markets.

The pipeline project promises major business and major technological challenges for many key industries in Canada and the United States—steel, construction equipment, valves and turbine compressors, ditchers and welding machines, engineering and consulting services. It should therefore come as no surprise that the Canadian government hopes to use the Alaska Highway project as a lever for convincing more foreign companies to start working and manufacturing in Canada, using more Canadian talent instead of just Canadian raw resources.

On the planning boards in government offices, the Alaska Highway project is only one of a long list of expensive and increasingly technically difficult energy projects, including tidal power, oil sands extraction plants, heavy oil extraction and up-grading plants, more northern gas pipelines, LNG tanker transportation schemes, nuclear power (using the CANDU system developed in Canada), hydroelectric developments (including the use of DC transmission for long-distance power movement), offshore exploration, and coal gasification plants.

As with the Alaska Highway pipeline, the uncertainty surrounding these projects relates more to the availability of financing than of technical expertise. And the Canadian government says it is willing to invest in equity in such projects if necessary, if only to make sure the money is available. □



SPACE

Meteosat's turn

Judy Redfearn reports on Europe's latest satellite

METEOSAT, Europe's first satellite devoted entirely to watching the weather, was scheduled for launch this week from Cape Canaveral into a geostationary orbit 35,900 km above the equator. But a valve in the second stage of its Thor Delta 2914 launcher developed a leak last weekend. NASA has announced that the launch will be delayed, until it can replace either the launcher or the valve.

When Meteosat finally arrives in its planned orbit it will take images of the earth's surface and cloud cover both in the visible and infrared which will be used by European meteorological services to improve their long range weather forecasting. The idea to build a geostationary meteorological satellite was included in the European Space Agency's (ESA) optional application programme in 1971.

Meteosat will, in fact, be only the

first of several European meteorological satellites. The second is already approved and should be ready for launch in the early 1980s and subsequent ones are under consideration. Meteosat therefore has a dual role: it is partly pre-operational, designed to prove a satellite system, but it is also an important scientific satellite in its own right.

From its position in space, Meteosat will relay back to earth meteorological data on most of Europe, the whole of Africa and the Middle East. Together with four other geostationary satellites positioned symmetrically above the equator, two American (Goes), one Soviet (GOMS), and one Japanese (GMS), it will make weather monitoring of the whole globe possible between latitudes $+50^\circ$ and -50° . The five satellites will be taking part in the first global experiment, running from the end of 1978 to the end of 1979, of the Global Atmospheric Research Programme (GARP).

The Japanese GMS was launched last July, and the USA already has

three Goes satellites up—two it will use and the third it is storing in space as a spare. But the Soviet Union's satellite, due for launch in about a year's time, is unlikely to be ready because of technical difficulties. NASA's precaution in keeping a stand-by ready may yet save the first global experiment, though no decision on how to replace the Soviet satellite has been taken.

A feature distinguishing Meteosat from other satellites is the way in which the data it generates is processed. Because of the enormous data volume—two images every half hour—all processing must be done in real time. The facility set up especially to do this and to disseminate information to the users is at the European Space Operations Centre (ESOC) at Darmstadt. After the images have been corrected for distortion at Darmstadt, they are relayed back to the satellite and then on to the users.

Should Meteosat itself fail, however, there will be another chance. As with the Orbital Test Satellite, ESA took out insurance for \$16 million to cover costs of a replacement launcher and integration of a second satellite. □

SWEDEN

Fälldin's energy puzzle

Wendy Barnaby, in Stockholm, updates nuclear developments in Sweden

CONSERVATION groups in Sweden recently succeeded in stopping a long-disputed application to mine the bulk of Europe's uranium. The application, which the mining company LKAB had previously withdrawn and re-presented taking more account of environmental considerations, was to mine 200 tons of uranium a year for ten years from an area about 350 km south-west of Stockholm, where the uranium deposits are estimated to be the largest in the West in the price range of \$10–15 a pound (1968 prices). The local government body used its right to veto any project involving such widespread damage to the environment which, it maintains, is rich in cultural treasures. A spokesman for the company said that it would now begin discussions with the government and that this could lead to a research and development project going ahead, with a small amount of mining to sustain it.

But the action taken over the LKAB application is only one of the pieces, a relatively small piece, of the country's

energy puzzle. There are many others that have yet to be fitted in. At the centre of the government's difficulties is the interpretation of the law which specifies the conditions under which the building of reactors may continue. Under the law, the owners of any reactor being planned or under construction must present the government with concrete proposals of the 'completely safe' storage of unprocessed waste or of highly-radioactive waste, if the spent fuel is to be reprocessed. Reprocessing agreements must also have been concluded before the government gives permission for the reactor to be built.

Although the owners of Barsebäck 2 and Ringhals 1 and 2 reactors have concluded agreements with the French Cogema company for reprocessing spent fuel, there is some doubt that the government will recognise the agreements as fulfilling the conditions of the law. This is because of a report prepared jointly by a committee of Cogema's trade unions, employers and safety representatives, which demanded that security at the plant be improved to meet national and international standards and itemised 47 points on which improvements in security and operations in general could be made.

Critics here say that the spirit of the Swedish law demands reprocessing agreements which will guarantee workable, reliable reprocessing. The reactor owners maintain that their responsibility under the law stops with the signing of legal agreements, and that the security matters to be taken care of at the plant are Cogema's business, not theirs. They are happy, they say, to trust the Frenchmen's technical abilities to solve the problems at their end. Overshadowing the entire deal is a query about the American government's attitude. There is no guarantee that the Americans, who supply Sweden with its enriched uranium and can veto the export of spent fuel from Sweden for reprocessing, will allow it to go ahead.

The government must soon decide which interpretation of the law it is to favour. It has given one reprocessing agreement to the state Nuclear Power Inspectorate for comments, and these are expected to be published late in November. The government's decision is expected in December.

December will also see the first of two 'security reports' being presented to the government by the nuclear power industry. The first one will deal with storage of reprocessed spent fuel, and will maintain that safe storage is technically possible. The second, to

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PROFILE

Chris Sherwell on Sir Hermann Bondi

An effective use of oil

HERMANN BONDI followed an auspicious precedent in British government when he left the Ministry of Defence to take over as chief scientist at the Department of Energy seven weeks ago. Before him, only Solly Zuckerman and Alan Cottrell had ever held the position of chief scientist in two separate departments of state—though they moved from Defence to the Cabinet Office, and in the days before the Rothschild reorganisation gave the institution its present form.

The appointment itself, when it finally came, was no surprise to anyone who had bothered to read the newspapers. But there was a gap before it was announced while the minor storm generated by the departure of Bondi's predecessor, Walter Marshall, subsided. The gap which Bondi himself left at Defence remains, and though names are being canvassed, no indication of who is in the running is officially available.

Bondi came to Energy with a remarkable record. Schooled in Vienna, where he was born 58 years ago, he went to Trinity College, Cambridge, and then became what was bureaucratically called a 'temporary experimental officer' in the Admiralty from 1942–45. In 1954, after holding a Trinity fellowship and lecturing for the best part of eight years, he was appointed to the chair of mathematics at King's College, London. Then in 1967 a leave of absence allowed him to become director general of the European Space Research Organisation. He stayed until 1971, when he became chief scientist at the Ministry of Defence.

The experience at Defence colours his approach in his new post. He regards the issue of nuclear proliferation as particularly important, for example. But as he puts it, "Things have to be looked at with a certain order of priority". Short term problems like proliferation must be checked before long term ones can be tackled. If the world is going to blow itself up first, he is not too worried about, say, the threat of a 'greenhouse effect' from over-use of fossil fuel.

Not that he has no views on the greenhouse effect. He says the US National Academy of Sciences report published earlier this year has been read as "more definite" than he reads it. If atmospheric oxygen comes from living plants making use of carbon

dioxide, he asks, and rising carbon dioxide levels can encourage plants to grow and so stabilise the rise, might not preservation of the most active plants in the best parts of the world be better than a restriction on the use of coal? It is a question for the long term, Bondi says simply, which will be of no interest at all unless we attempt to ensure our survival in the short term.

He is reticent about giving his own views on such immediate issues as maintaining the breeder option or Britain's choice of reactor. He stresses that energy as a whole, and not just nuclear power, is capital intensive and expensive, the turnover of the energy industries being about the same as the defence and education budgets combined. As he sees it, R&D in alternative energy technologies must be fostered in case conventional sources become unacceptable, unavailable or too expensive, but the judgment of likely fuel costs is complex.

One economic consideration which puzzles him is the effect of the oil price increase four years ago. The developed countries initially responded by saying they would maintain their economic growth and replace oil with nuclear power. That led to colossal predictions for the growth of nuclear power, says Bondi. "But the calculations were completely bogus". The fuel crisis stopped growth, and nuclear forecasts slumped. Instead of energy prices rising relative to other things, inflation and a lack of growth caused fuel costs in many fields to become a smaller share of the total than they were. "So if I'm asked whether a project for an alternative energy source would become economic if the real price of fuel doubled, I must ask if engineering costs will go up equally disproportionately, and whether we'll be back where we started".

As Bondi sees it, a rise in fuel costs, rather like a failed harvest in developing countries, has to mean a substantial drop in the standard of living. But developed countries have been totally unwilling to accept this. Is there an appreciation of this within government? "I preach it", Bondi replies with a smile. He is as aware as anyone of the problems governments face, as he shows when he allows his thoughts about costs to stray to current issues. The difficulty



Sir Hermann Bondi

when it comes to choosing a reactor or investing in alternative energy sources, he says, is not really a lack of money, but spending it sensibly and responsibly—not whether to purchase, but what to purchase.

So how should a chief scientist be seen? "Like any scientist", Bondi says, "the chief scientist has a particular standing when he gives a judgment on scientific matters. But he wouldn't be a responsible citizen if he didn't have a judgment on other matters as well, although he has no more claim to authority on these than anybody else". Bondi regards the important element in the chief scientist's task as "making clear what the scientific issues are, within the context of other questions".

He is clear why he came to Energy. First of all he was asked, and he has an appetite for change. Another attraction was that it was controversial. But there is a third aspect, which has to do with his time at Defence. He enjoyed his time there, he says, and they enjoyed having him. He rejects completely any suggestion that they wanted to see him go. But the contribution of somebody from outside springs from the person's different background and different way of thinking. "He treads water for a while, learns to understand the issues and how to handle the machine. Then he can make a distinct contribution. But after some years he loses the advantages an outside background is supposed to confer".

He has now applied the lesson to himself. At Defence, he says, he had become part of the furniture. "I was with my fourth secretary of state, my third permanent secretary, and my sixth chief of defence staff". Continuity has its own contribution to make, he says. "But I'm not convinced that I was bringing to it as much as I had done, say, two years ago, though"—and this with a twinkle—"I knew very well how to put a drop of oil into the machine". He now has more oil, and a smaller machine.

IN BRIEF

Reactor choice soon

Consultations held by Britain's energy minister, Mr Anthony Wedgwood Benn, on the country's choice of thermal nuclear reactor, are drawing to a close now that most submissions from interested parties on the subject have been received. A decision is expected before Christmas.

The latest submission on the issue has come from the Trades Union Congress (TUC), representing Britain's labour movement. Its fuel and power industries committee wants the immediate ordering of an AGR station with another later, and a major effort to work up a detailed and acceptable PWR design before a decision on a third station is made. The committee also supports the construction of a demonstration breeder reactor and of expanded reprocessing facilities at Windscale.

Last month the country's two main electricity boards, the CEBG and SSEB, submitted their views to the Department of Energy. The department has also published virtually in full the report of an assessment of the three reactor types under consideration by the National Nuclear Corporation.

ACORD members named

The chairman of the UK Science Research Council (SRC), Professor Geoffrey Allen, has stepped into another appointment recently vacated by his predecessor at SRC, Sir Sam Edwards: membership of the Advisory Council on Research and Development for fuel and power (ACORD). Dr Joseph Gibson, the board member for science at the National Coal Board, joins ACORD as NCB representative.

ACORD, with 10 longer-serving members drawn entirely from industry,

will shortly have to take a serious decision: how to advise Benn on the Severn Barrage schemes for tidal power, which will help to energise Britain but may radically alter the ecology of her greatest estuary.

SA's nuclear optimism

With the controversy over South Africa's nuclear intentions still volatile, the president of the South African atomic energy board, Dr Ampie Roux, has said that if the United States cuts off supplies of nuclear fuel for the Koeberg nuclear station north of Cape Town, his country's uranium enrichment process could ensure supplies within a few years of the station's start-up date in the early 1980s. Dr Roux also said that South African uranium output would increase to make her the West's second largest producer after the United States.

SOCIOBIOLOGY seeks to explain that human social behaviour has a genetic, and, therefore, an evolutionary background, which has long been obvious for other orders of animals, especially the *Hymenoptera*. Against the acceptance of this concept is the fact that many people dislike being compared with monkeys and object even more to being told that they resemble insects.

I am becoming increasingly nervous about my behaviour being the result of millions of years of evolution, a conclusion I have to reach if I embrace the teachings of sociobiology. The thought puts me on my mettle; I should try not to let my genes down. My behaviour is a rather poor illustration of a progressive evolutionary process, but the sociobiologists say that social evolution may indeed be occasionally reversible. Other nagging questions assail me: should I obey my impulses because these promptings are the result of inherited patterns of mental processes? Or should I suppress these impulses in the biological interests of benefiting my group? Or should I try to shape up so as to be a survival machine for my genes, which, says Richard Dawkins, "swarm in huge colonies, safe inside gigantic lumbering robots, sealed off from the outside world . . . their preservation is the ultimate rationale of our existence . . ."?

In any case, the subject of sociobiology merits attention because Dick Lewontin dislikes it, and he is a man of strong convictions on important issues. Moreover, perhaps sociobiology could be an interesting chal-

lenge to the pan-selectionist school of evolutionists, who object to the proposal, made by the so-called 'neutralists', that amino acid replacements in homologous proteins of different

On sociobiology



THOMAS H. JUKES

species are often changes that have no effect on the properties of the protein, but are incorporated by genetic drift. The neutral model also says that many mutations are deleterious and are rejected by natural selection, and a few become accepted by a species because they are advantageous.

The opposing view states that each amino acid in a protein must have a unique survival value in the phenotype of the organism. Would ethologists extend this general principle to

behavioural phenomena? Perhaps the idea of neutral hereditary changes could be extended to explain certain habits that have little to commend them, or it could even account for harmless quirks that have no particular bearing on our survival as a species. A leading sociobiologist reassuringly says that only about 10% to 15% of human social behaviour is genetically based, but I don't know what has led him to such a quantitative assessment. The same author says, "The hypothalamic-limbic complex automatically denies . . . logical reduction by replacing it with feelings of guilt and altruism". In other words, our emotions, which are part of our inheritance, prevent us from reaching conclusions based on logic. This is a facile way of disarming criticism, of course.

It seems to me that sociobiology aggravates its opponents by the ingenuity with which it produces explanations to make observations fit a theory. To a considerable extent, pan-selectionism has done the same by finding an adaptive advantage for each phenotypic trait. We are told that even the hair-pattern on our backs was selected by evolution in our ancestors to help shed the rain as they crouched on the branch of a tree during a tropical storm.

Perhaps those who disbelieve and spurn sociobiological teachings may be responding to a genetic trait. The struggle against pre-ordained fate could in itself have a survival value. This struggle may include the rejection of theories that tell us we are in the grip of determinism imposed by our genes.

news and views

Gametes of malaria parasites

from F. E. G. Cox

It has always been one of the major aims of parasitology to maintain parasites in culture and to minimise as far as possible the use of human subjects or laboratory animals. A year ago, the possibility of growing all stages of the human malaria parasite, *Plasmodium falciparum*, in culture was brought a step nearer when Trager and Jensen (*Science* **193**, 673; 1976) described the continuous cultivation of the blood-inhabiting asexual stages of this parasite in a simple medium. The next stage in the malaria life cycle is the development of gametocytes (the stages that infect mosquitoes) from the asexual stages in the blood and in this issue of *Nature* (page 240) Carter and Beach describe how this can be achieved *in vitro*. The early stages of *P. falciparum* that invade red cells can develop into either asexual stages, a process which takes about 18–24 h, or into gametocytes which takes about 10 days (Smalley *Nature* **264**, 271; 1976). It was the development of the asexual stages that Trager and Jensen achieved. Carter and Beach observed that in cultures of *P. falciparum* maintained in

Trager's medium, without the dilution that is usually carried out, obvious immature gametocytes were present after the expected 10 days. The problem was how to bring these stages to maturity. This was achieved by adding 50% human serum instead of the usual 10% to the cultures and after 8 days morphologically mature gametocytes were present. The next step was to confirm that these gametocytes were functionally mature by transforming them into gametes and this was done by suspending the gametocytes in foetal calf serum at pH 8.0 or human serum at pH 8.2. Trager's culture medium requires a pH of 7.3–7.4 for the growth of asexual stages so it is essential to change the pH in order to bring about these transformations.

The gametocytes and gametes produced by Carter and Beach look normal in every way and occur in similar proportions of male to female (1:3.5 compared with 1:4) to those in humans. There is no reason to doubt that they should be able to infect mosquitoes. This means that it should now be possible to complete the asexual and sexual cycles of *P. falciparum* without the need for a mammalian

host. It will next be necessary to grow the liver stages of the parasite *in vitro* but this should not present any insuperable difficulties. In the meantime, numerous experiments on the infectivity of *P. falciparum* to mosquitoes have become possible.

The question as to how long the gametocytes of *P. falciparum* are infective to mosquitoes is an interesting one. Hawking, Wilson and Gammage (*Trans. R. Soc. trop. Med. Hyg.* **65**, 549; 1971) calculated that gametocytes were produced in a circadian pattern and that they would be infective for only a short period. Bray, McCrae and Smalley (*Int. J. Parasit.* **6**, 399; 1976) on the other hand, found no evidence for a circadian rhythm and Smalley and Sinden (*Parasitology* **74**, 1; 1977) showed that, in humans, gametocytes are infective for 2 weeks or more. The observations of Carter and Beach suggest that gametocytes can mature to gametes for several days at least and this supports the observations of Smalley and Sinden. The technique itself will be useful in resolving problems of this kind in future. It should also be possible to test the effects of drugs on gametocytes—a hitherto relatively unexplored field. □

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Coevolution of *Calvaria* and the dodo

from Robert M. May

MUTUALISTIC associations, in which two species interact in such a way that each benefits from the presence of the other, are fascinating for several reasons. For the theoretician, they are increasingly shedding light on the interplay between the long sweep of evolution and the immediate dynamical effects of population interactions (for example, King, Gallaher & Levin *J. theor. Biol.* **53**, 263; 1975). For the naturalist, and even for the pure aesthete, they provide some of nature's

richest wonders: the homes and food that many tropical acacias supply for their protective ants; the nectar supplied by flowering plants as the price for pollination; the almost erotic coupling between many tropical orchids and their obligate bee pollinators.

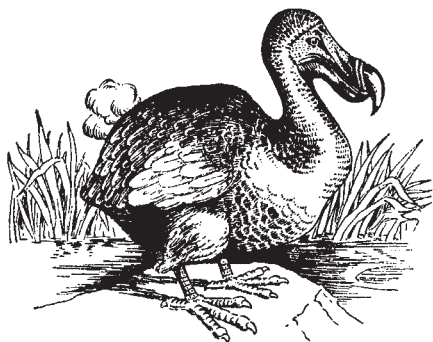
One class of mutualistic interactions we should all be grateful for is that between fruit-producing plants and the animals that disperse their seeds. The fleshy part of the fruit is the cost the plant pays to induce animals (typically birds) to eat the fruit; the associated benefit is that the seeds, having taken some time to pass through the animals,

are spread widely. The fruits we eat are the direct or horticulturally-improved outcome of such coevolutionary cost-benefit calculations.

In systems of this kind, a major design problem is producing a seed that can survive the passage through the digestive tract of the fruit-eating animal. A partial answer is to speed the passage of the seed; hence the mildly laxative effect of many fruits (notably prunes).

All this brings us, rather surprisingly, to the dodo. This large, flightless bird was endemic to the 700-square-mile island of Mauritius. Its disappearance by 1681 is probably the most familiar

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The Dodo (*Raphus cucullatus*)

example of an extinction directly attributable to man: 'The Dodo used to walk around/And take the sun and air./The sun warms his native ground—/The Dodo is not there!/The voice which used to squawk and squeak/Is now forever dumb—/Yet may you see his bones and beak/All in the Mu-se-um.' (Belloc, *The Bad Child's Book of Beasts*, Dover reprint, 1961; see also Hachisuka, *The Dodo and Kindred Birds*, Witherby, 1953). Stanley Temple (*Science* **197**, 885; 1977) has drawn attention to the fact that the large monoecious tree *Calvaria major*, which according to historical records was once abundant and exploited for lumber on Mauritius, also seems to have undergone a trauma dating back to the dodo's demise. There were by 1973 only 13 *Calvaria major* trees surviving in the remnant native forests of the island, and all 13 were estimated to be more than 300 years old. No younger specimens are known to exist, even though the surviving trees produce well-formed, apparently fertile seeds each year. None of these seeds now germinate naturally, even if planted in nursery conditions.

Temple notes that, although there are several examples where the elimination of a plant species has been deleterious for an associated animal species, the *Calvaria*-dodo system may be the first documented instance where disappearance of the animal partner has doomed a mutualistic plant species. The mechanism suggested by Temple is that the seed inside the *Calvaria* fruit developed a coat (endocarp) thick and strong enough to resist crushing in the powerful gizzard of the dodo. As a result, 'these specialised, thick-walled pits could withstand ingestion by dodos, but the seeds within were unable to germinate' without the coat first being battered and abraded by the stones in the dodo's gizzard.

In support of this hypothesis, Temple observes that the *Calvaria* fruits are today consumed by some frugivorous animals, notably a parakeet and a flying fox, but that none is large

enough to eat the fruit whole, and thus disperse the seed. The dodo was large enough, and therefore it was presumably the customer for whom the tree designed its fruit. There is fossil evidence that dodos ate *Calvaria* fruit. Furthermore, measurements of the crushing forces generated by the stones in the gizzards of granivorous birds from several taxonomic groups (ranging in body weight from 0.8 to 3.2 kg) give a formula which, when applied to the 12-kg dodo, suggest crushing forces of around 10^4 kg m^{-2} in its gizzard. *Calvaria* pits are strong enough to withstand these forces, but only just: Temple suggests that once the pit had been sufficiently abraded to reduce its size by 30%, the seed would be crushed. But by then some of the seeds would have passed through the dodo, albeit after a battering which would weaken the endocarp and facilitate germination of the infant plant within.

As an experimental test of his ideas, Temple force-fed *Calvaria* seeds to turkeys, whose gizzards generate crushing forces around $4,000 \text{ kg m}^{-2}$, fairly close to those inferred for dodos. Of 17 seeds, seven were crushed. The remaining 10 were either regurgitated or defaecated, some after up to 6 days. These 10 seeds were planted in nursery conditions, and three subsequently germinated. Temple concludes that 'these may well have been the first *Calvaria* seeds to germinate in more than 300 years.' □

More evidence for a closed Universe from QSOs

from T. Kiang

FURTHER evidence that quasars (QSOs) can provide information on the nature of the Universe comes from the work of Chinese astronomers. Their work complements the recent observation of the ultraviolet spectrum of the QSO 3C273 by Davidsen *et al.* (*Nature*, **269**, 203, 1977; *News and Views* **269**, 195; 1977). The Chinese astronomers have constructed Hubble diagrams for various subsets of radio QSOs using a different luminosity indicator from Davidsen *et al.*, but come to essentially the same conclusion.

It has long been realised that one of the best means to decide whether the Universe is open (the declaration para-

meter q_0 less than $\frac{1}{2}$ and the Universe expanding for ever) or closed (q_0 greater than $\frac{1}{2}$ and the Universe eventually falling back on itself) is by means of the Hubble diagram, in which the redshift z is plotted against the apparent magnitude m . (The redshift is the reddening of the light from stars and galaxies due to their recession). But the relations predicted by different cosmologies become distinguishable only when the redshifts extend beyond the present limit (~ 0.5) for the galaxies. That is why the discovery of QSOs with much higher redshifts brought high hopes. However, these hopes were soon dashed, for the first Hubble diagrams for QSOs looked like pure scatter diagrams, showing hardly any trend whatever. Subsequently, some astronomers took the view that the QSO redshifts are 'intrinsic' and not 'cosmological' at all.

Most astronomers, however, held to the view that these redshifts are at least largely a distance effect, and that the featureless Hubble diagram is due to the sample being a mixture of many different types of object. In support of this view, Setti and Woltjer (*Astrophys. J. Lett.* **181**, L61; 1973) showed that, if the sample is restricted to QSOs with steep radio spectra, then the scatter is much reduced, and a trend of the expected kind can be seen. Similarly, Stannard (*Nature* **236**, 295; 1973) found that the same thing happens if only objects with flat spectra are considered. But in these diagrams, there is still a large dispersion, which is attributed to spread in luminosity within each type. Further progress thus awaits the identification of some 'luminosity indicator', and that is where J. A. Baldwin's recent demonstration of the correlation between $\text{L}\alpha$ intensity and continuum luminosity comes in (*Astrophys. J.* **214**, 679; 1977). Using the equivalent width of $\text{L}\alpha$ as a luminosity indicator, Davidson *et al.* identified five quasars with z between 2.3 and 3.4 which are presumably as luminous as 3C273 at $z = 0.158$. Here at last is a Hubble diagram for objects of a limited luminosity range, and this fact is probably more significant than the particular result they obtained ($q_0 \sim +1$) which the authors are quick to point out, critically depends on what one assumes for the galactic reddening in the direction of 3C273.

The diagram by Davidsen *et al.* is not the first one of its kind. In a paper in the Chinese journal *Acta astronomica sinica* (**17**, No. 2, 134; 1977; English translation in *Chinese Astronomy*, **1**, No. 2, in the press) an entirely different luminosity indicator was used and Hubble diagrams for

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various subsets of radio QSOs constructed, leading to a value of $q_0 = +1.38$. The authors of this paper are Fang Li-zhi, Zhou You-yuan, Cheng Fu-zhen and Chu Yao-quan from the University of Science and Technology of China—names quite unknown in the West.

Confining their attention to those QSOs with resolved radio components, these authors propose that the linear separation D between the components be used as a luminosity indicator. (They eventually back up this suggestion by actually deriving a value for the rate of change of the optical luminosity with D). Since D is not directly observable, a rather subtle statistical argument was used in getting plausible estimates of D . Starting with observed angular separation θ and redshift z , and assuming a distance-redshift relation $r = r(z)$, one calculates the projected distance $r\theta$, which differs from D by a projection factor $\sin \psi$, say. Now, in an isotropic distribution of directions in space, $\sin \psi$ is concentrated near its large end value of 1. The authors argue that what one can do here is to take only those quasars with $r\theta$ values at or close to the observed maximum $r\theta$ value at the given z , and then simply set $D = r\theta$ for such objects. In this way, from a sample of some 90 radio quasars with known θ and z , they picked out 26 for which such indicative values of D can be assigned. Separate Hubble diagrams were then constructed for various ranges in D .

Rather tight correlations consistently appear in these diagrams, which typically have 10 data points and a scatter of about 0.6 magnitudes. But the linear regression coefficient of m upon $\log z$ departs significantly from 5, which means that the classical Hubble relation $r(z) \propto z$ is no longer valid. In a self-consistent series of calculations, the authors then found that, in order to recover a slope of 5, the regression analysis must be made between m and $\log(z - 0.19z^2)$. This is equivalent to saying that the correct distance-redshift relation is of the form $r(z) \propto z - 0.19z^2$, and that is how the formally precise value of $q_0 = +1.38$ was obtained, because in relativistic models, the coefficient of z^2 here is equal to $\frac{1}{2}(1 - q_0)$.

Although the true uncertainty in this determination must be very much larger than the formal value implies, it is probably not as large as in the derivation by Davidsen *et al.*, because the sample is larger and the sample points more evenly distributed. The two results complement each other in that one deals with flat-spectrum objects and the other, mainly steep spectrum objects. They agree in saying

that the Universe is probably closed.

On the other hand, because of consistent failure in detecting sufficient mass in one form or other, astronomers in recent years have become more and more disposed towards the opposite alternative of an open Universe—this, for example, was very much the general feeling in the recent Symposium on Large-Scale Structure of the Universe, held at Tallinn, Estonia.

The determination of q_0 tacitly assumes that no change of luminosity has taken place in time. Hence the challenge now is to find a physically plausible model of luminosity evolution that will account for both a low mass density and a high value of q_0 .

The evaluation of q_0 is only one of several results obtained by Fang Li-zhi *et al.* Many astrophysicists may find their result that the optical luminosity of a quasar decreases by 2.3 magnitudes for every megaparsec increase in the linear separation between its radio components to be even more important. □

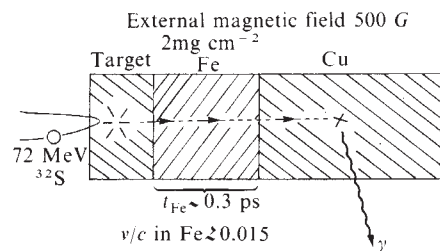
Magnetic moments of short-lived nuclear states

from P. E. Hodgson

A NEW method of measuring the magnetic moments of nuclear states of very short lifetime has recently been developed by N. Benczer-Koller and colleagues at Rutgers University, and some preliminary results have just been reported at the recent conference on nuclear structure held in Tokyo in September. They have applied the method to several isotopes of iron, nickel and zinc and obtain results that are important for testing nuclear models.

In this method, a 72 MeV beam of ^{32}S ions hits the target material, which is deposited on a 2 mg cm^{-2} layer of magnetised iron backed by 10 mg cm^{-2} copper, as shown in the figure. Some of the target nuclei are excited, and these recoil through the iron and stop in the copper, where they decay by gamma emission. The energy of the gamma ray identifies the state of excitation of the recoiling target nucleus.

The gamma rays are detected in coincidence with the ^{32}S ions recoiling in the direction opposite to that of the incident beam. This ensures that the knocked-on target nuclei pass perpendicularly through the magnetised iron so that the length of their path in that



material is accurately known. As the target nuclei pass through the magnetised iron their magnetic moments precess and the angle of precession is measured by the rotation of the angular distribution of the de-excitation gamma rays, $\Delta \theta \sim g \int B dt$. The value of $\int B dt$ and the constant of proportionality are determined by a subsidiary experiment using ions of known magnetic moment, so that a measurement of $\Delta \theta$ enables g to be found. It is sufficient for practical purposes to use an empirical relation describing the dependence of B on the velocity of the ion; this is quite different from the theoretical relation, but this lack of understanding of the process does not affect the determination of the magnetic moments.

As an example of the results obtained, the magnetic moment of the lowest 2^+ state of ^{54}Fe , that has a mean life of only 1.4 ps, is found to be 1.68 ± 0.38 . Magnetic moments have also been determined for the lowest 2^+ state of ^{58}Fe , and for the same states of four isotopes of nickel and four isotopes of zinc. In some cases the magnetic moments were already known from other work, and the new and old values are in good agreement.

This method has many advantages over those used previously. The high magnetic field inside a ferromagnet enables the magnetic moments of states with lifetimes in the ps region to be measured, whereas the methods using laboratory magnetic fields are limited to states with lifetimes in the ns region. Since the ion is moving rapidly through the magnetised iron and does not stop there the results do not depend on the rate of energy loss at low velocities. This is a major source of uncertainty in the methods in which the ions come to rest in the iron. Furthermore, $\Delta \theta$ is independent of the lifetime of the ion providing it is sufficient for the ion to pass through the magnetised region.

The magnetic moments of excited states can be calculated from the shell model, and provide a searching test of its validity. The new results for the nickel isotopes disagree with shell model calculations that take no account of core excitation, but are in good accord with newer calculations that include $f_{7/2}$ core excitation. □

P. E. Hodgson is a lecturer in Nuclear Physics in the University of Oxford.

Epiphyte or parasite?

from Peter D. Moore

It is not difficult to define the difference between an epiphyte and a parasite; the former depends on another organism simply for support whereas the latter exploits its host in a manner which is normally beneficial to the parasite but detrimental to the host. Neat definitions, however, are not always easy to apply in complex field situations. Take, for example, the relationship between the robust, brown, fucoid alga *Ascophyllum nodosum*, which is abundant in the intertidal zone of most of the more sheltered shores around the British Isles, and the small red alga, *Polysiphonia lanosa*, which is almost invariably associated with it. The red alga adheres strongly to its host, since its thallus actually penetrates the tissues of *Ascophyllum*. Where the biomass of the host is greatest, there one finds the greatest density of *Polysiphonia* also. Here is a perfect situation in which the epiphyte/parasite debate can be staged.

One can see certain advantages in the epiphytic habit in this type of algal community. Elevation during immersion provides obvious benefits, for the mass of floating fronds of the brown alga, buoyed up by air bladders, must cast a considerable degree of shade upon any bottom-dwelling algae. At the same time, close association with *Ascophyllum* could make life more comfortable during the periods of immersion when extremes of variation in temperature, relative humidity, salinity and light intensity would be dampened by the presence and protection of a mucilaginous mass of limp fronds. Simple epiphytism would not, therefore, be an unreasonable expectation in such a circumstance.

The possibility that *Polysiphonia* is more than an innocuous passenger demands examination, however, for the attachment of the red algal thallus to its host seems remarkably secure for a species association which is found only where wave action is very slight. An examination of the anatomy of the attachment region by Rawlence and Taylor (*Can. J. Bot.* **48**, 607; 1970) showed that rhizoids of the 'epiphyte' penetrated deeply into the thallus of *Ascophyllum*.

Citharel (*C. r. Séanc. hebdom. Acad. Sci. Paris, Ser. D*, **274**, 1094; 1972) used a physiological approach to the problem, considering that, since no true epiphyte would take up metabolites

from its host, any demonstration of transport of reduced carbon from the brown alga to the red would provide grounds for an accusation of hemiparasitism. Citharel chose glutamic acid as the most likely vehicle of carbon transport, since this is the most abundant free amino acid in the *Ascophyllum* thallus. He injected ^{14}C labelled glutamic acid into the thallus of *Ascophyllum* and observed its accumulation in *Polysiphonia* within 12 h. He concluded that, contrary to the opinion of such algal authorities as Fritch, *Polysiphonia* should be considered a partial parasite. The demonstration of such a transfer does not, however, necessarily infer that the red alga is dependent upon *Ascophyllum* as a carbon source, indeed Harlin and Craigie have argued that it is not (*J. Phycol.* **11**, 109; 1975).

A further complication has now been brought to light by translocation experiments by Turner and Evans (*New Phytol.* **79**, 363; 1977). They supplied ^{14}C -labelled bicarbonate ions to *Ascophyllum* tissues and studied translocation of the label to parts of the plant isolated from the initial supply, and also into tissues of *Polysiphonia* attached to the host plant. In this way they hoped to overcome two possible objections to the experiments of Citharel, first that the labelled compound supplied by him was not in fact the product of photosynthetic activity in *Ascophyllum* and, second, that transport of metabolite could occur by secretion into the surrounding medium and reabsorption by *Polysiphonia*, or even by way of the mucilage surrounding the species. The outcome of Turner and Evans' experiments demonstrated that there was no translocation of the label in the tissues of *Ascophyllum* either with or without attached *Polysiphonia*. This means that if *Polysiphonia* is acting parasitically it can only exploit the cells in the immediate vicinity of the rhizoids, for the lack of translocation in the host will preclude any wider effect of such parasitism. They also demonstrated that *Polysiphonia* was able to accumulate ^{14}C from labelled bicarbonate in the surrounding medium, confirming the independent carbon assimilation of the red alga, but they further showed that the 'epiphyte' was able to take up and use exogenously supplied labelled glucose.

So the question of whether or not *Polysiphonia lanosa* is a true epiphyte of *Ascophyllum nodosum* still remains unanswered. What is now clear is that if *Polysiphonia* depends upon its host

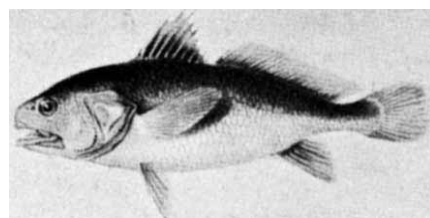
for more than support in a physical sense, it will be limited in its success by the lack of movement of metabolites in the host tissue and will be dependent on a local exploitation of cells or the absorption of materials secreted into the surrounding medium. As Turner and Evans point out, however, one cannot be sure that carbon is the sole object of any parasitism which occurs. Perhaps some other element is being sought, or even an organic growth factor. □

Indo-Pacific drums reviewed

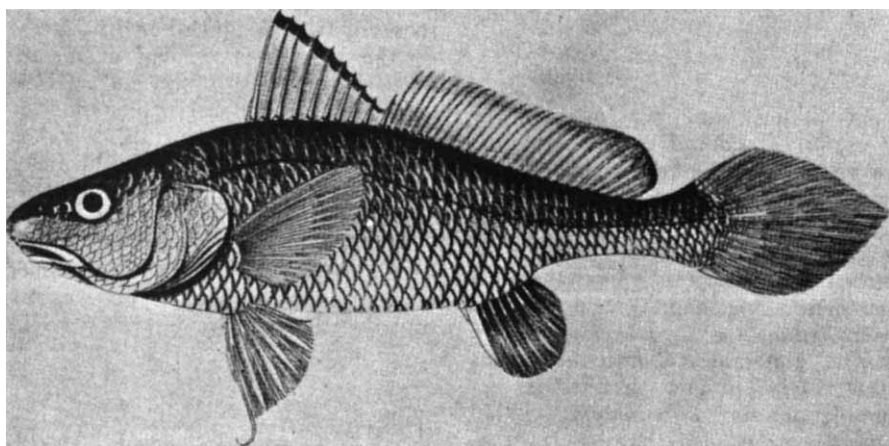
from a Correspondent

THE drums or croakers are fishes of very great commercial importance throughout tropical seas especially in shallow regions and where large rivers join the sea. They derive their common names from their habit of making loud noises of one kind and another; indeed it is claimed that native fishermen of South-east Asia only set their nets when their leader has heard the fish approaching the area. Because of their economic importance fishery workers in various countries have made numerous studies of their biology and aspects of their life history, and some have attempted taxonomic studies to establish some kind of phylogeny as well as a valid nomenclature. Notable amongst these was the work of Y. T. Chu, Y. L. Lo, and H. L. Wu, *A study of the classification of the sciaenoid fishes of China, with descriptions of new genera and species*, Shanghai Fisheries College, 1963, with a short English summary. Chu *et al.* produced a work of fundamental importance in which they placed considerable emphasis in classification on the structure of the otoliths and the swimbladder. Their synthesis proved to be a major landmark in understanding this group of fishes although necessarily it was largely confined to Chinese waters (where their commercial importance is very great).

However, their work has now been amplified and refined by a major revision, *The sciaenid fishes (croakers or drums) of the Indo-West-Pacific by*



Johnius (Johnnieops) dussumieri (Cuvier) (Cuvier *Le règne animal*, 1840).



Johnius coitor (Hamilton *An account of the fishes of the Ganges*, 1822).

Ethelwynn Trewavas (*Trans. zool. Soc. Lond.* 33, 253; 1977). Trewavas has expanded the area covered by major revisionary studies quite logically to include the less well known, but equally important, Indian Ocean species. Much of the new synthesis is founded on the form and structure of the swimbladder and otoliths for, having extended knowledge of these two organs, Trewavas finds them well found as major characters.

The degree of development of the swimbladder is a fascinating study and six main types are identified among the Indo-Pacific species. At its least elaborate the swimbladder is a simple large gas-filled sac with no appendages. Relatively few species (mostly Atlantic in their distribution) possess this form. Other types have diverticula running from the anterior end of the swimbladder and variously directed backwards or forwards (in one group running forwards into the head to underlie the skull), but in the subfamily Otolithinae the swimbladder is decorated with paired series of between 9 and 53 diverticula, mostly elegantly branched. In one tribe, Collichthyini, within this subfamily the diverticula are so developed that posteriorly their limbs enwrap the viscera, a situation which is paralleled in a distantly related West African family.

The large size of the otoliths of the sciaenid fishes is well known, both to science and to folklore (a necklace made of otoliths of a drum was found in a Californian Indian archaeological site), and names such as Otolithinae used within the group indicate that they have long been accepted as characteristic. Trewavas has extended the previous application of this feature to the taxonomy of the group, describing several new types and rationalising the nomenclature of the parts of the sagitta used by previous workers.

The net result of the analysis of otolith and swimbladder structure as

well as conventional taxonomic methods, such as fin ray, vertebra and scale counts, and morphological differences, is to produce a fundamentally important taxonomic work. In all, 65 species are recognised, grouped in 27 genera and 10 tribes. Each species is adequately described, with diagnostic

characters singled out, and basic synonymies are presented, with brief discussion on the synonymy. Keys are provided to all taxa as are diagnoses of families and genera. In total it presents fisheries workers in the Indian and West Pacific Oceans with a basic source for the identification of these important food fishes, one which was badly needed to judge from the synonymies presented.

It is peculiarly apt that these fishes which are well known for their sonic abilities have now been classified basically by the highly specific complex patterns of the swimbladder and the otolith and inner ear. It is generally accepted that the former is a sound-producing organ, of quality in this case, while the latter is concerned with sound reception. Living as they do in shallow tropical seas, especially in the clouded waters close to and within estuaries, the sciaenids have developed a sensitive auditory system which enables them to keep together and presumably to distinguish each other from other related species. □

Animal migration at Tübingen

from Michael A. Bookman

A Symposium on Animal Migration, Navigation, and Homing was held in Tübingen, FRG from 12–20 August, 1977, in celebration of the 500th anniversary of the University of Tübingen.

ALTHOUGH the Sun can be used by many animals as a source of compass information, the ability to form a bright image on the retina and simultaneously derive accurate bearings without retinal damage remains puzzling. J. D. Pettigrew (California Institute of Technology) presented an interesting model linking the avian pecten oculi to solar orientation. Light scattered from the Sun's image on one edge of the retina would be interrupted by the pecten, which would cast a well-defined linear shadow over the temporal fovea adjacent to the image of the horizon. Peripheral imaging of the Sun prevents serious retinal damage while information is relayed to orientation-selective edge detectors in the visual wulst of the brain. With the potential for precise determination of the Sun's trajectory, some people were undoubtedly reminded of the 'rejected'

theories of bicoordinate solar navigation. An ingenious camera device that can be mounted on a pigeon's head to record the free-flying line of sight was described by K.-L. Köhler (Fürth, FRG). Investigation of orientation strategies with such a technique may aid the experimental evaluation of Pettigrew's model.

The sensory capabilities of homing pigeons were further reported on by M. L. Kriethen (Cornell University), J. D. Delius and J. Emmerton (Ruhr Universität, Bochum) and M. A. Bookman (Massachusetts Institute of Technology). In the laboratory, pigeons have demonstrated sensitivities to polarised light, ultraviolet light, small changes in barometric pressure, infrasound, and magnetic fields. Conditioning and electrophysiological experiments suggest that regional specialisation of the retina may optimise perception of polarised light. Sensitivity to ultraviolet light seems to result from the non-absorbance of the pigeon optical apparatus. L. J. M. Leask (Clarendon Laboratory, Oxford) proposed that the retina may also be involved in the detection of magnetic fields by a process of optical pumping and anisotropic decay of triplet states. Improved characterisation of magnetic responses, such as those by W. Wiltschko (Universität Frankfurt) with

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migratory birds, will help in the formulation and testing of possible sensory mechanisms. The influence of magnetic fields on orientation was further documented by C. Walcott (State University of New York, Stony Brook), who reported on the persistent disorientation of pigeons released near natural magnetic anomalies. The extent to which many of these sensory capabilities are made use of in navigation is unknown, although hypotheses abound.

The role of wind-carried odours in pigeon homing was discussed by F. Papi and S. Benvenuti (Università di Pisa), K. Schmidt-Koenig and J. Kiepenheuer (Universität Tübingen), and R. F. Hartwick (James Cook University, Queensland). Most investigators acknowledged that in some circumstances odours could provide directional cues; however, most data do not support a central role for odours in pigeon homing. In a later discussion, W. T. Keeton (Cornell) reasoned that just as a hierarchy of redundant cues seems to supply compass information, a similar hierarchy is probably used to obtain map information. Therefore, research should not aim to uncover 'the' navigational scheme, because several schemes are likely to be used in different conditions.

Odours, however, clearly occupy a central role in the spawning of salmon. A. D. Hasler and A. Scholz (University of Wisconsin, Madison) described a remarkable experiment in which hatchery-raised salmon were exposed to chemically scented water for 1 month before their release into Lake Michigan. During the subsequent spawning season, small streams were artificially scented with the same compounds. Imprinted salmon were then recovered in large numbers from the appropriately scented stream.

The sensitivity of marine sharks, skates, and rays to voltage gradients on the order of $0.01 \mu\text{V cm}^{-1}$ was described by A. J. Kalmijn (Woods Hole Oceanographic Institute). In the laboratory, these fish oriented to magnetic fields, presumably by responding to local electric fields induced by swimming through the magnetic field. Theoretically, fish could monitor natural voltage gradients found within ocean currents to obtain compass information and determine the direction of water flow.

In experiments with hatchling sea turtles, M. Mrosovsky (University of Toronto) demonstrated that movement from the nest to the water's edge is governed by a phototropic response to an integrative right-left balancing of retinal and tectal stimulation. L. C. Ireland (Oakland University, Rochester) used ultrasonic transmitters to track hatchling turtles on the initial portion of their maiden ocean voyage.

As yearlings, the turtles reach distant feeding grounds. He concluded that the initial directional stimulus was simply 'away from land', which perhaps represented an extension of nest-to-water movement.

Whether migratory birds are capable of true bicoordinate navigation or predominantly utilise only simpler forms of compass orientation remains controversial. T. C. and J. M. Williams (Swarthmore College) reported that successful migrants moving between North and South America seem to maintain a Southeast heading during their entire autumn migration, and would therefore not require a bicoordinate system. S. A. Gauthreaux (Clemson University, South Carolina) observed inland daytime flights of nocturnal migrants which are felt to correct for night-time drift. These flights suggest that some nocturnal migrants may actually navigate during the early daylight hours. A new release and tracking procedure for migratory birds using balloons and radar was developed by S. T. Emlen and N. J. Demong (Cornell). Although complex, this technique combines experimental control with near-natural flight conditions. Initial experiments showed that sparrows were able to select meaningful headings when released under cloud layers that obscured celestial cues. □



A hundred years ago

Bees killed by *Tritoma*

In a friend's garden here where there are quantities of *Tritomas* or "red-hot-pokers," hundreds of bees have been this year destroyed by them. The honey produced by the flower is very abundant, and the bees enter the tube of the corolla to get at it; but the tube, which is only just large enough at the mouth, tapers gradually, and so the bee gets wedged in and cannot extricate itself. I saw numbers so caught, some in the fresh flower, while others remained in the completely withered and decaying blossoms. Perhaps it may be due to the fine warm days we have had this autumn, inducing the bees to work too late after our native honey-producing flowers have been destroyed by the wet and frosts; or is it a regular thing which happens every year? If so bee-keepers should discourage the *Tritoma*, or set to work to select varieties with flowers large enough not to kill their bees.

ALFRED R. WALLACE

From *Nature* 17, 15 November, 45; 1877.

Plasma lipoprotein structure

from Angelo M. Scanu

A workshop on Lipoprotein Structure was held at the University of Chicago on 8-9 October, 1977.

DURING the past 10 years, many studies have been carried out on the structure of circulating lipoproteins, relying primarily on physical and chemical techniques. The workshop provided an assessment of the current state of knowledge on the subject.

The discussion on small-angle X-ray scattering was focused mainly on the low-density lipoproteins (LDL), where controversy still exists on the state of organisation of their surface components. The recent studies by V. Luzzati *et al.* (Gif-sur-Yvette, France) have led investigators to postulate the existence of 'bumps' or 'spikes', which were attributed to protein units protruding from the surface of the particles. The investigations of P. Laggner *et al.* (Graz, Austria), in turn, although yielding essentially similar X-ray results, were interpreted as being compatible with a surface having no convolutions. The organisation of the LDL core also came under sharp scrutiny. There was agreement on the presence, in this core, of cholesteryl esters and triglycerides in varying proportions; the concentric smectic lamellar organisation of the cholesteryl esters suggested by the studies of D. Atkinson and G. Shipley (Boston), was disputed, however, by Luzzati, who proposed an alternative organised micellar structure without, however, giving details. Agreement seems to exist on the notion that triglycerides influence the core organisation of cholesteryl esters; this view appears well documented by the results of studies on the LDL of animals with hyperlipidemias secondary to the administration of cholesterol-supplemented dietary fats.

Contrary to the rather extensive studies performed with small-angle X-ray scattering, the work on plasma lipoproteins carried out by neutron diffraction (H. B. Stuhmann, FRG) has been comparatively modest. The validity of the method was recognised, however, and further applications were anticipated with suitable replacement of some of the LDL constituents by their deuterated counterparts. An elegant study of the naturally occurring quasi-crystalline egg yolk lipoprotein system was presented by L. Banaszak (St. Louis University, St. Louis), who has reconstructed a two-fold symmetry model from electron micrographs of negatively-stained preparations.

Differential scanning calorimetry is proving its usefulness in the study of plasma lipoproteins. A systematic study of the thermal behaviour of LDL, HDL, and related systems was reported by D. Small (Boston), who obtained information on the phase behaviour of cholesteryl esters in the core of LDL particles. He also investigated the thermal stability of HDL, particularly that of its major protein, apolipoprotein A-I. R. Biltonen (University of Virginia, Charlottesville), using differential scanning calorimetry, estimated the statistical distribution of cluster sizes in phospholipid bilayers, and H. Pownall (University of Texas, Houston) applied microcalorimetric techniques to examine the association between apolipoproteins and lipid vesicles. Fluorescence spectroscopy was used by A. Jonas (University of Illinois, Urbana) in a study of lipoproteins and apoprotein-phospholipid-cholesterol recombinants; she proposed a bilayer structural model conceptually similar to that derived from X-ray and thermocalorimetric data. Pownall described the kinetics and mechanisms of lipid-amphiphile association, whereas Smith discussed an interesting application of an extrinsic fluorescence probe (pyrene), to the study of cholesterol exchange between lipoproteins using stopped flow kinetics.

The current status of ^{13}C -NMR spectroscopy as applied to studies of the structure of plasma lipoproteins was summarised by E. Cordes (Indiana University, Bloomington). The results presented indicated that this technique is valuable in the study of the dynamics and phase transitions of surface and core lipids, particularly in combination with the analysis of model systems. J. Morrisett (Houston) reported results of his ^{13}C and ^{31}P -NMR spectroscopic studies on lipoprotein-X, an abnormal lipoprotein found in cholestasis. T. Glonek (University of Chicago) discussed the use of a chelated manganese paramagnetic ion to probe the accessibility and surface distribution of the phospholipids in both LDL and HDL.

One session was devoted to an examination of the mechanism of phospholipase A_2 action and its use as a probe of the lipoprotein surface. F. Kézdy and colleagues (Chicago) found that the kinetic behaviour of this enzyme is similar for all of the major lipoprotein classes investigated; he therefore suggested that all of the phospholipase A_2 -hydrolysable phospholipids represent a single pool at the surface of these particles. M. Wells (University of Arizona, Tucson) presented evidence that the physical state of the substrate influences the specificity of phospholipase A_2 and pointed at the limited information available at present on the mechanism of action of

Red hot sea

from Peter J. Smith

It is as certain as anything can ever be in the Earth sciences that the Red Sea is a region of very young seafloor spreading. Some of the evidence for this view comes from geothermal studies, which is only to be expected, although it comes as a surprise to be reminded by Girdler and Evans (*Geophys. J.* **51**, 245; 1977) that as recently as 1970 there were no more than 17 heat flow values available, 5 of which were only estimates from borehole temperature measurements. Since 1970, however, the quantity of data has increased fivefold, and Girdler and Evans have now been able to draw a much better, though still far from complete, picture of Red Sea geothermal characteristics.

The average heat flow for measurements made within 5 km of the Red Sea's deep water axis is 467 mW m^{-2} , or about eight times the world average of 59 mW m^{-2} , although individual values rise to more than 56 times the world average. Over the next 5-km interval the average then drops dramatically to 89 mW m^{-2} , presumably because of hydrothermal circulation; but it rises to 140 mW m^{-2} and 165 mW m^{-2} over succeeding 20 km intervals, settling to about 111 mW m^{-2} (still about twice the world mean) at distances of 50–170 km.

Thus heat flow is high not only in the axial zone but throughout the whole of the Red Sea. Indeed, both heat flow and temperatures (100°C at

depths of less than 2 km) are so high along the edges of the sea that Girdler and Evans see geothermal heat as a potential source of power for coastal towns. But only 7 of the 86 individual heat flow values published by the end of 1975 lie below the world average; and as all come from the vicinity of the deep axial trough where heat flow is typically high, they would seem to reflect only transient thermal effects such as sediment slumping, or perhaps their positions above the downgoing limbs of hydrothermal circuits.

Sedimentary movement and hydrothermal circulation in the axial zone probably also explain the high variability of heat flow near the centre of the Red Sea. According to Girdler and Styles (*Nature*, **247**, 7; 1974) there have been at least two phases of seafloor spreading, the lithosphere produced by the second intruding and heating the older lithosphere and overlying evaporites; and although some people would dispute this precise interpretation, there seems every reason to suppose that evaporite movement has resulted from very recent spreading. Such complexity makes detailed interpretation of heat flow data difficult; but the general geothermal picture is evidently entirely consistent with the Red Sea as a young spreading zone. □

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the enzyme. L. Smith (Houston) discussed the mode of activation of a purified milk lipoprotein lipase preparation by fragments of apolipoprotein C-II, the specific activator of the enzyme, and identified regions considered to be specific for activation. He discussed a similar approach with lecithin-cholesterol acyl transferase, in which he used fragments of apolipoprotein C-I, one of the activators of the enzyme.

With regard to apolipoproteins, W. Fitch (University of Wisconsin, Madison) presented an elaborate study on structural predictions based on primary sequence data. The findings led to the recognition of striking internal homologies in apolipoprotein A-I, considered to originate from duplication and crossing over of an ancestral gene coding for an 11-residue repeat. A computer search for amphipathic helices in apolipoprotein was discussed by J. Segrest (University of Alabama, Birmingham), whose results support the hypothesis that these segments might

be responsible for lipid binding. G. Fasman (Brandeis University, Waltham) provided a lucid analysis of the role of β -turns in protein structure and of the experimental methods used for their detection. The importance of β -turns in apolipoprotein structure emerged from work by investigators at the University of Chicago, who presented the first full-scale space-filling model of human HDL $_2$, based on available experimental data and on work in progress on the properties of the A apolipoproteins at the air-water interface. One major conclusion from these investigations was that all of the predicted structures (α -helix, random coil, and β -turns) in apolipoprotein A-I and A-II are amphipathic, and that this unique property enables them to occupy predictable areas at the HDL surface. □

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review article

X-ray burst sources

Walter H. G. Lewin & Paul C. Joss*

More than 30 X-ray burst sources have been discovered in the past 2 years, and information on the observational properties of the burst emission is accumulating rapidly. The physical mechanism responsible for the bursts is so far undetermined, but the most promising models are based on the accretion of matter onto a compact object of Stellar mass.

X-RAY bursts from a source in the globular cluster NGC6624 were discovered by Grindlay and Heise¹ in December 1975. Earlier, events had been reported that were believed to be hard X-ray bursts of cosmic origin². However, after lengthy discussions between one of us (W.H.G.L.) and Dr Melioranski, we believe that these bursts and others reported by the same group^{3,4} are not of cosmic origin but are probably caused by the Earth's magnetosphere.

In 1976 a further 19 X-ray burst sources were discovered, mostly by SAS-3 and OSO-8 (refs 5–7). To date (15 September 1977) the total number of burst sources is in excess of 30; they are strung along the galactic equator (Fig. 1) and the majority of them are located within $\sim 35^\circ$ of the galactic centre⁷. At least two (but probably several more) are located in globular clusters^{8–12}, and three burst sources have been tentatively identified with faint blue stars^{13–15}. One of these identifications, proposed by McClintock, is almost certainly correct^{13,16}.

A typical burst rises in less than a few seconds and lasts several seconds to minutes. In most bursts, the lower energy flux persists longer than the higher energy flux (Fig. 2). In at least three cases^{17–19}, the time-dependent burst spectra can be fitted well by blackbody emission from a source with an effective radius of ~ 10 km. If this interpretation is correct, there is good reason to believe that the burst sources are neutron stars or black holes of stellar mass.

The bursts are sometimes emitted at approximately regular intervals of from hours to days. At least eight burst sources emit a persistent (though variable) flux of X rays. No persistent emission has been detected from at least two burst sources^{5, 20, 21}.

The mechanism which produces X-ray bursts is not yet known. There is little evidence to suggest that the burst sources are in binary systems, though many workers favour this idea. No proposed model has yet given a complete explanation for the behaviour of burst sources.

Galactic distribution

If we adopt as an operational definition for X-ray bursts⁵: (1) rise time less than a few seconds, (2) duration from a few seconds to a few minutes, and (3) recurrence, then eighteen burst sources have been reported to date^{7,12} (15 September 1977). Their positions are shown in Fig. 1. If we accept single burst-like events for which only the first two criteria were met, seven burst sources can be added (dashed lines and horizontal bars in Fig. 1). At least five more burst sources (not shown in Fig. 1) have been observed with SAS-3; their positions are very uncertain but they are probably all located within $\sim 30^\circ$ of the galactic centre.

Many other burst-like events of cosmic origin that we omit have been reported in the literature; they are not known to meet either the rise time or the recurrence criterion. In this context we mention the intriguing results of the Los Alamos group^{22–24}. It is very likely that some of the many events observed by them were X-ray bursts of the kind discussed here.

The burst sources are spread along the galactic equator and cluster near low galactic longitudes ($|l| < 35^\circ$). The distribution is different from that of the known X-ray sources with 'steady' emission^{5,7}. Eight burst sources (Table 1) have been identified with sources of steady emission (more will undoubtedly follow). This suggests that the burst sources form a subset of the steady X-ray emitters^{5,7}. No steady emission has been observed from at least two sources⁵.

Burst flux and recurrence

The observed peak intensities in bursts are typically $\sim 10^{-8}$ to $\sim 10^{-7}$ erg cm⁻² s⁻¹. At source distances of ~ 10 kpc, this would imply a peak luminosity of $\sim 10^{38}$ – 10^{39} erg s⁻¹.

The total (integrated) flux of individual bursts differs by about 1–2 orders of magnitude from source to source. In general, the total flux of individual bursts from a given source does not change appreciably over a time scale of many burst intervals^{5,6}.

Aside from the short rise times (less than a few seconds) and the longer decay times (several seconds to minutes), an important characteristic of X-ray bursts is their recurrence (not reported for γ -ray bursts). As first reported by Clark and his associates²⁵, the recurrence is sometimes regular though not strictly periodic. The most regular burst intervals have been observed from MXB1659–29. During October 1976 the burst intervals from this source wandered slowly from 2.0 to 2.6 h, and the burst arrival times were often predictable to within several minutes^{5,20}. Again, during the recent worldwide coordinated burst observations²⁶ in June and July 1977, SAS-3 detected a series of 17 bursts (intervals ~ 2.5 h) with arrival times often (but not always) predictable to within 5 min. It may not be a coincidence that MXB1659–29, which is the most 'regular' of all burst sources, does not emit a detectable flux of 'steady' X-rays^{5,20}.

The apparent regularities in burst occurrence for several sources can persist for days^{5,6}. During recent SAS-3 observations, however, we found that many sources that were believed to be regular actually become irregular on time scales of days to weeks. The most striking examples of highly irregular burst sources are MXB1837+05 (refs 27–29) and MXB1735–44 (ref. 30 and unpublished SAS-3 results); their burst intervals can vary from ~ 1 h to ~ 2 d.

The fact that most known burst sources have intervals from hours to days is probably the result of a selection effect due to the limitations of today's X-ray observatories; the longer the

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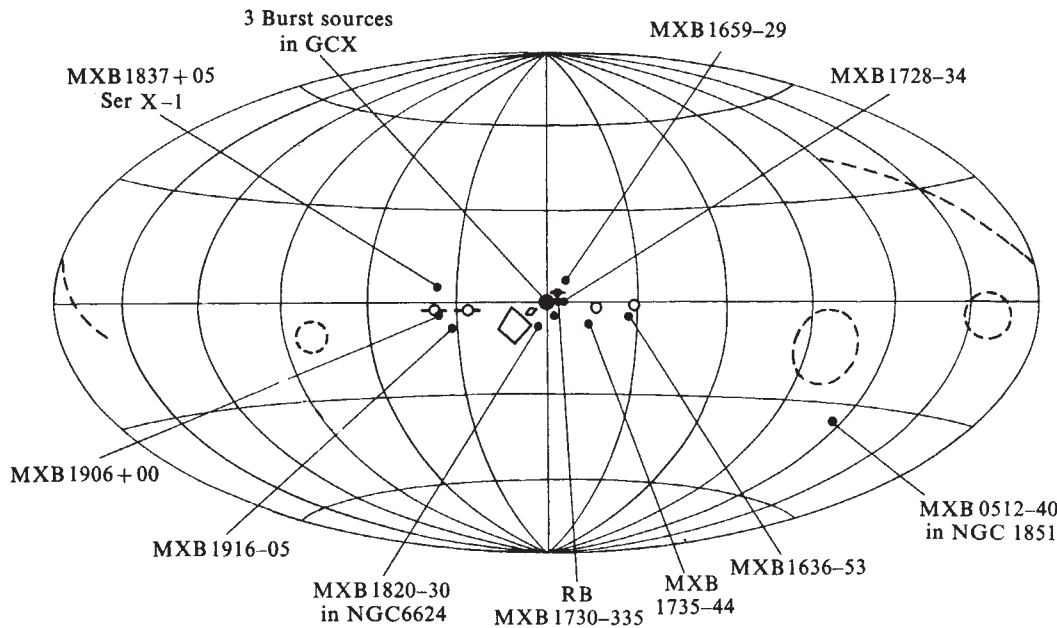


Fig. 1 Sky map (galactic coordinates) of 18 X-ray burst sources which meet the three burst criteria (see text). Seven additional sources are shown for which the recurrence criterion of the bursts has not (yet?) been met. They are indicated with dashed lines (four sources) or with horizontal bars (three sources). Open error regions have areas $\geq 3 \text{ deg}^2$. The dashed line near the galactic anticentre represents an error box of $\sim 120^\circ \times 2^\circ$. The figure contains only burst sources reported before 15 September 1977. There are at least five additional known sources with poorly determined positions (see text). The names of some of the best studied burst sources are shown.

burst intervals, the smaller the chance of detection. It is likely that sources will be discovered with considerably longer burst intervals (weeks, months, years?). There are at present seven sources (Fig. 1) from which only one burst has been observed, yet some of these sources were observed continuously for many days. This may already be an indication that some of them have burst intervals in excess of several days. It is expected that in the era of the space shuttle, sensitive X-ray detectors will be flown with both large fields of view and the capability of determining source positions accurately. Such instruments would be ideal for the study of fast transient phenomena such as bursts.

It is thought that most burst sources have burst-active and inactive periods^{5,6}. The most striking examples are MXB1820-30 (in NGC6624), MXB1730-335 (the rapid burster), MXB 1743-28, and a burst source in Norma (refs. 5, 6, 21, 30-36). There is no indication that the periods of activity come at regular intervals, but this may well be the case for some sources. A possible (so far undetected) periodicity in the burst patterns or in the active and inactive periods could, in principle, uncover an orbital period of a binary system.

Burst profiles

Bursts exhibit a large variety of shapes. There are bursts with spikes, peaks, bumps, shoulders and tails, and some show all these features⁵. Bursts from different sources usually look very different (Fig. 2). Bursts from one source, however, usually seem quite similar. Several burst sources have their own characteristic idiosyncracies, and we can often (but not always) tell by the burst profile from which source a burst came (Fig. 2). All eight bursts detected from MXB1743-29 (at ~ 35 -h intervals) showed two (sometimes three) distinct peaks at energies above 6 keV (ref. 33). Bursts from MXB1735-44 are usually narrow, lasting only 4-6 s³⁰. Bursts from MXB1636-53 and MXB1728-34 have fast initial decays (a few seconds) followed by long gradual decays at low energies^{18,19,37}. There are cases, however, where the bursts from one source look very different. Bursts from MXB1837+05 show great variation²⁹ which may be related to the irregularities in the burst intervals. Normally, bursts from MXB1728-34 occur quite regularly with intervals that wander slowly from ~ 4 to $\sim 8 \text{ h}$ ^{18,37}. But, in June 1977, during the worldwide coordinated burst observations²⁶, only one burst was observed in 54 h, and the profile of this burst was very peculiar. During observations in July 1977 the bursts from MXB1728-34 again occurred regularly and seemed 'normal'. There is some observational evidence that

bursts which occur 'out of sequence' in a quasi-regular series have a different appearance from the other bursts in the series. This effect has been reported in bursts from MXB1636-53, MXB1916-05 and MXB1730-335 (the rapid burster) (refs. 19, 21, 38 and unpublished SAS-3 results).

Clark *et al.*³¹ have observed 22 bursts from MXB1820-30 (in NGC6624). The burst intervals slowly wandered from $\sim 3.4 \text{ h}$ to $\sim 2.2 \text{ h}$. The burst tails, which were clearly visible at the beginning of the observations, vanished gradually and were absent from the last few bursts. The burst activity came to a halt while the persistent emission from the source continued to increase.

Burst spectra

Significant spectral changes occur during bursts. Typically the spectra soften considerably during burst decay (Fig. 2 and refs. 5, 6). The best examples are the bursts from MXB1743-29 (ref. 33), MXB1906+00 (ref. 39), MXB1735-44 (ref. 30 and unpublished SAS-3 results), MXB1728-34 (refs 18 and 37), MXB1636-53 (refs 19 and 40), MXB1916-05 (refs 41-43), MXB0512-40 (in NGC1851, ref. 10 and G. W. Clark, personal communication) and a source near $l^{\text{II}} \sim 356^\circ.4$, $b^{\text{II}} \sim 2^\circ.3$ (ref. 17). Spectral hardening during burst decay has only been observed in bursts from MXB1820-30 (refs 8, 25). Spectral hardening during the burst rise is quite common, however, and has been observed in bursts from MXB1728-34, MXB1636-53, MXB1820-30, MXB0512-40 and in a single burst from a source near $l^{\text{II}} \sim 356^\circ.4$, $b^{\text{II}} \sim 2^\circ.3$. MXB1837+05 is special, as some bursts harden during the rise and others soften²⁹.

Swank *et al.*¹⁷ and Hoffman *et al.*^{18,19} have found good blackbody fits to the spectra of bursts from three burst sources (MXB1728-34, MXB1636-53 and a source near $l^{\text{II}} \sim 356^\circ.4$, $b^{\text{II}} \sim 2^\circ.3$). During the early part of the bursts, the temperature increases to $\sim 3 \times 10^7 \text{ K}$. A cooling is then observed which continues for several minutes, after which there is no longer any detectable flux. If indeed the burst emission is blackbody radiation, the effective radius R of the emitting region can be calculated. Swank *et al.*¹⁷ find that $R \sim 100d \text{ km}$ during the first 20 s of the burst and $R \sim 15d \text{ km}$ for the remainder of the burst, where d is the source distance in units of 10 kpc. Hoffman *et al.*^{18,19} find for both MXB1728-34 and MXB1636-53 that $R \sim 10d \text{ km}$. This size remains approximately constant throughout the bursts. These results suggest that, if the source distances are $\sim 10 \text{ kpc}$, then the bursts are produced by a neutron star or a black hole of stellar mass¹⁷⁻¹⁹.

Hoffman *et al.*¹⁹ have discussed 'typical' bursts in terms of

two components: (1) an initial pulse which rises and falls sharply in a few seconds and is wider at higher energies, followed by (2) a long decay whose spectrum softens with time as the burst intensity gradually decreases (Fig. 2). They made the interesting observation that the initial pulse appears to be highly absorbed at low energies (1–3 keV) when a burst in a quasi-regular series arrives out of sequence (too early) (ref. 19 and unpublished SAS-3 results).

Steady emission

It is quite common for a burst source to emit a persistent (but variable) flux of X-rays^{5,6}. As shown in Table 1, high 'steady' fluxes ($\sim 1/4$ that of the Crab Nebula) are observed from MXB1636–53 (refs 40, 44, 45), MXB1728–34 (refs 18 and 37), MXB1735–44 (ref. 30) and MXB1837+05 (refs 27–29). No steady flux has been observed from either MXB1730–335 (ref. 31) or MXB1659–29 (ref. 5).

Table 1 lists eight burst sources and the accurate SAS-3 positions of their associated steady sources (refs 46 and 47 and J. G. Jernigan, personal communication). It is highly probable that all associations in Table 1 are correct; the probability (based on the positional coincidences) that any one of them is wrong is $\sim 1\%$. It has been suggested³³ that the three burst sources near the galactic centre produce a steady emission that may account for part of the emission of GCX (ref. 54). There are a few more burst sources for which a candidate steady source has been proposed^{12, 34, 48, 53}.

The ratio of time-averaged luminosity of the steady emission to that of the burst emission varies from $\lesssim 2$ (rapid burster) to ~ 250 (ref. 19). If we exclude the rapid burster, the lowest value is < 25 for MXB1659–29 (unpublished SAS-3 results). These ratios are important in testing nuclear-flash models of X-ray bursts (see section on theoretical models).

Optical identifications

There are optical candidates^{13–16} for three burst sources (MXB1837+05, MXB1636–53 and MXB1735–44). All three are faint (~ 18 mag) blue stars. At least one star, recently suggested by McClintock, is almost certainly a correct identification^{13, 16}.

Very accurate positions for the steady sources that produce bursts have recently been reported by the Ariel 5 group in Birmingham⁵¹ and the SAS-3 group^{46, 47}. It is expected that as a result more optical identifications will soon follow.

The burst sources MXB1820–30 and MXB0512–40 are located in the globular clusters NGC6624 (refs 8, 25, 31) and NGC1851 (refs 10 and 11), respectively. The rapid burster²¹ (MXB1730–335) is almost certainly located in 'Liller's' globular cluster (refs 5, 6, 9, 55), and a burst source with a positional accuracy of ~ 1 deg² may be located in the globular cluster NGC6441 (ref. 12). Thus, a very high fraction of the seven known X-ray emitting globular clusters⁵⁶ also emit bursts, and it is expected that more burst sources will be found in globular clusters. The large majority of burst sources, however, (Fig. 1) are not located in visible globular clusters^{5–7}.

Spectra and character of associated steady sources

The spectra of the persistent X-ray emission of burst sources do not fit a blackbody model and are almost always softer than the mean burst-spectra⁵ (integrated over the first ~ 10 s of the burst). The only exception is 4U1915–05 (MXB1916–05), for which a hard spectrum ($kT > 35$ keV) has been reported for the steady emission⁴³.

At Ostriker's suggestion⁵⁷, Cominsky (personal communication) has compared 'colour-colour' plots of the steady X-ray emission from eight burst sources (the hard source 4U1915–05 was not included) with that of other types of X-ray source (five globular clusters, seven strong galactic centre sources, one pulsating low-mass binary, two non-pulsating low-mass binaries, seven high-mass binaries, and two black hole candidates). She concludes that the colour-colour plots are similar for: (1) the burst sources (steady emission), (2) the globular cluster X-ray sources, (3) the strong galactic centre sources, and (4) the non-pulsating low-mass binaries (Sco X-1 and Cyg X-2). The colour-colour plots, however, are different for the high-mass binaries, the pulsating low-mass binary (Her X-1) and the two black hole candidates (Cyg X-1 and Cir X-1). This suggests that the first four categories may have very similar mechanisms for producing X-rays and that they may well all be binaries. But, if the burst sources are binaries, one may wonder why none of them shows any evidence of their binary character. On the other hand, it is very difficult to deduce a possible binary membership from X-ray data if no pulsations or eclipses are observed (as in Sco X-1 and Cyg X-2) and if the approximate binary period is not already known (for example, from optical observations). It may well be that the possible binary character of X-ray burst sources will first be found in optical data. It is expected that the optical

Table 1 Steady X-ray sources from which bursts have been observed*

Burst source	Steady source	Positions of steady sources from SAS-3 (refs. 46, 47 and J. G. Jernigan, personal communication)						Error circle radius†	Intensity (Uhuru counts s ⁻¹ ; ref. 48)		Refs.
		RA (1950)	Dec. (1950)	h	min	s	°		Maximum	Maximum/minimum	
MXB0512–40 (in NGC1851)	2S0512–400 2A0512–399 4U0513–40	05	12	29	–40	05	53	20"	18	3	10, 11, 48–50
MXB1636–53 (blue star)	2S1636–536 4U1636–53	16	36	57.6	–53	39	21	20"	250	2	14, 19, 40, 45, 47, 48, 51
MXB1728–34	2S1728–337 4U1728–33	17	28	39.6	–33	47	52	30"	150	5	5, 6, 18, 37, 47, 48, 51, 52
MXB1735–44 (blue star!)	2S1735–444 4U1735–44	17	35	19.5	–44	25	22	20"	210	1.7	13, 16, 30, 47, 48, 51
MXB1820–30 (in NGC6624)	2S1820–303 4U1820–30	18	20	28.4	–30	23	14	20"	320	3	1, 5, 6, 8, 25, 31, 32, 48, 51
MXB1837+05 (blue star?)	2S1837+049 4U1837+04	18	37	29.8	04	59	23	20"	280	2	5, 6, 27–29, 46, 48
MXB1906+00	Ser X-1 2S1905+000 A1905+00 4U1857+01	19	05	54.9	00	05	37	35"	4.05 ± 1.1‡	–	5, 6, 39, 46, 48
MXB1916–05	2S1916–053 4U1915–05	19	57	02.9	11	34	14	20"	20	2	41–43, 46, 48

*These eight associations are quite reliable (see text). Four others^{12, 34, 48, 53} (not in this table) have been proposed but are uncertain.

†Each error circle is an approximate 90% confidence region.

‡Average intensity.

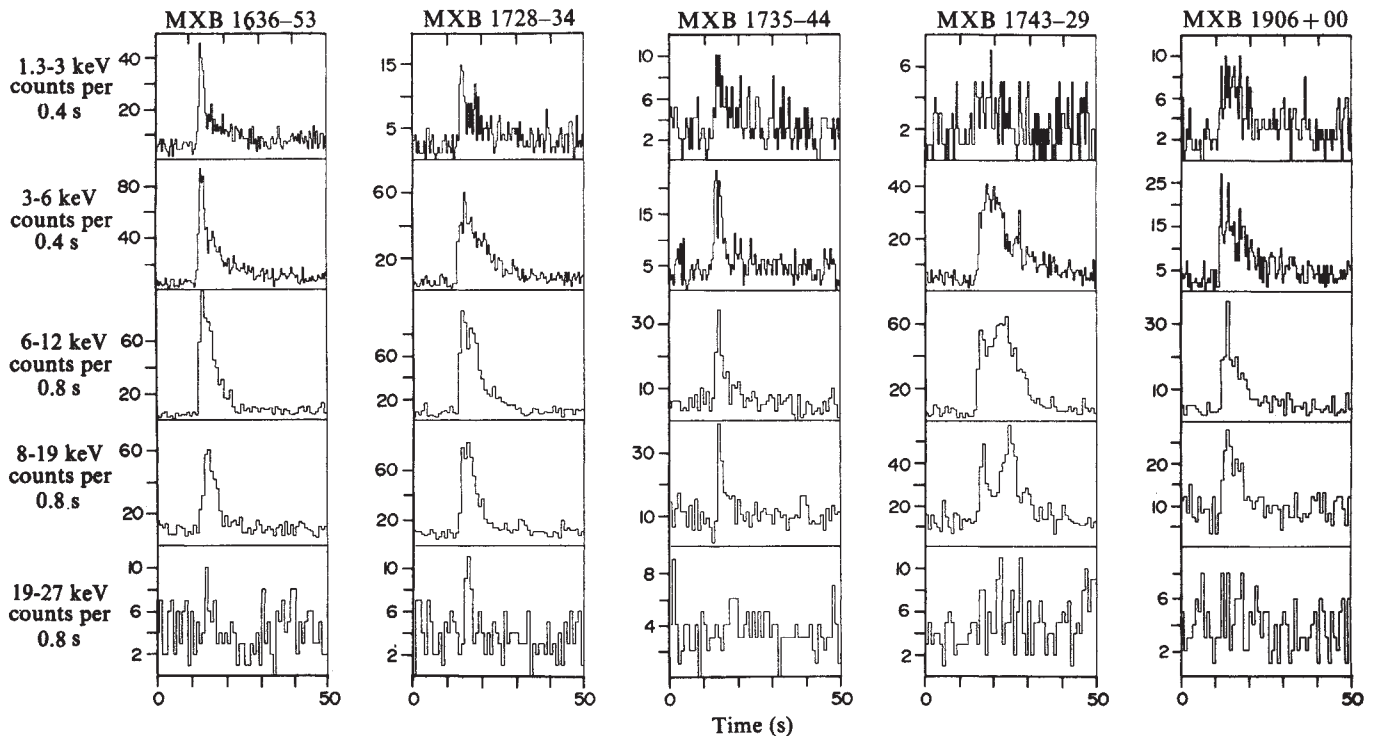


Fig. 2 Profiles (five energy channels) of bursts from five different sources (SAS-3 data). Note that in all cases the gradual decay (tail) persists longer at lower energies than at higher energies. The burst profiles are quite distinctive for each particular source (see text).

candidates¹³⁻¹⁶ will be carefully examined for evidence of binary characteristics.

The rapid burster

The rapid burster (MXB1730-335) is quite unlike all other burst sources²¹. It can produce up to 4,000 bursts per day⁵, the integrated energy E of individual bursts can vary by two orders of magnitude, and E is approximately proportional to the time Δt for the next burst to occur²¹. A more detailed analysis has shown that the linear relation breaks down at low burst energies, where the waiting time to the following burst is considerably longer than one would expect from a strictly linear relation⁵.

Occasionally, 'anomalous' bursts have been observed that occur more or less independently of the rapidly repetitive ('normal') burst sequence, grossly violating the $E-\Delta t$ relation³⁸. These 'anomalous' bursts in general rise more slowly, and their maximum luminosity is $\sim 1/3$ that of the maximum observed luminosity in 'normal' bursts³⁸.

The rapid burster is almost certainly located in a globular cluster^{9,55}, whose position is inside the small error circle (1.8 arc min radius) for the burst source^{6,51,58}. As noted above, no steady emission has been observed from this source; the ratio of time averaged steady flux to time averaged burst flux is $\lesssim 2$ (ref. 21), which is uniquely low^{5,6}. This source was burst-active in March-April 1976, in April-May 1977 and in September 1977 (refs 21, 30, 35, 36, 59). Bursts from this source have also been detected with Uhuru⁴⁸.

The time averaged burst luminosity varied significantly (by factors of $\sim 2-3$) during March and April 1977⁶. The latest SAS-3 results, obtained by H. Marshall (personal communication) show a hint of a period of about 0.8 d in the time averaged burst luminosity.

Comparison with γ -ray bursts

The most striking differences between X-ray bursts and γ -ray bursts⁶⁰ are: (1) X-ray burst sources cluster along the galactic equator whereas γ -ray burst sources do not; (2) unlike γ -ray bursts, X-ray bursts are repetitive on time scales of hours to

days; (3) the spectra of γ -ray bursts are much harder than those of X-ray bursts; and (4) the total flux density of a γ -ray burst is typically ~ 2 orders of magnitude higher than that of an X-ray burst. The γ -ray bursts cannot be the high-energy tails of normal X-ray bursts produced by nearby burst sources; the absence of recurrence excludes this possibility. However, some generic relation between γ -ray bursts and X-ray bursts cannot be excluded²⁴. It is interesting to note that a γ -ray burst (from galactic latitude $\sim -46^\circ$) observed from Apollo 16 shows a triple-peaked time structure⁶¹, which is remarkably similar to the structure in bursts from MXB1743-29 (Fig. 2 and ref. 33).

Theoretical models

Most speculations concerning the nature of X-ray burst sources fall into two broad categories: (1) instabilities in the accretion of matter onto a compact object (white dwarf, neutron star, or black hole), and (2) thermonuclear flashes in matter accreted onto the surface of a neutron star. Theoretical models based on these ideas are still rather primitive.

Among possible instabilities in accretion onto a compact object of roughly solar mass, most models are based on interchange instabilities at the Alfvén surface of a neutron star or white dwarf. Within this category, Lamb *et al.*⁶² have investigated the possibility of a thermal instability in spherical accretion onto a magnetised neutron star, while Svestka⁶³, Henriksen⁶⁴, Baan⁶⁵ and Joss and Rappaport⁶⁶ have discussed the possible role of orbital motion of the accreting matter in producing an instability. Wheeler⁶⁷ and Liang⁷⁰ have investigated instabilities within an accretion disk surrounding a compact object. These preliminary discussions are a promising start. The physics of the proposed instability mechanisms is very complex, however, and no completely satisfactory model has yet been established.

Grindlay and Gursky⁶⁸ and Grindlay⁶⁹ have presented the idea that the burst sources may be associated with massive ($\gtrsim 10^2 M_\odot$) black holes. A specific mechanism for producing the burst emission was suggested by Bahcall and Ostriker⁷⁰, who invoked a compact object in orbit about a massive black hole,

which interacts with an accretion disk that surrounds the hole. The latter idea is by now excluded; we now know that the burst intervals are often very irregular, unlike the highly periodic behaviour that one would expect from this model.

The introduction of massive black holes was largely motivated by the association of 3U1820-30, the earliest discovered burst source, with the globular cluster NGC6624 (ref. 8). This cluster is one of five globular clusters which have highly condensed cores and which contain sources of persistent X-ray emission⁶⁶. It had previously been suggested^{71,72} that these steady X-ray sources may be massive black holes undergoing accretion within the cluster cores. However, as more burst sources were discovered, it was found that the distribution of these sources is very different from the apparently spherical distribution of globular clusters about the galactic centre (Fig. 1 and refs 5-7). This fact, together with the absence of visible globular clusters in the direction of many burst sources, now makes it almost certain that the majority of burst sources are not located in globular clusters.

Another motivation for the massive black hole model was the spectral hardening observed during the decay in bursts from NGC6624 (refs 8, 25), which was ascribed to Compton scattering of the emitted X rays by a very hot ($\sim 10^9$ K) gas cloud that is gravitationally bound by a massive black hole⁶⁸. Canizares⁷³ has shown, however, that the observed hardening could be produced by scattering in a cooler cloud, which might be bound by a compact object of mass $\lesssim 5M_\odot$. Moreover, bursts from most other sources, including the one located in the globular cluster NGC1851, soften during their decay (Fig. 2, refs 5, 6 and G. W. Clark, personal communication). As pointed out earlier^{5,6}, it seems that little evidence is left to support the conjecture that X-ray bursts are associated with massive black holes.

Maraschi and Cavaliere⁷⁴ and Woosley and Taam⁷⁵ have suggested thermonuclear flashes in the surface layers of accreting neutron stars as a totally different alternative mechanism for the production of X-ray bursts. The gravitational energy released per unit mass of matter falling onto a neutron star of mass M and radius R will be $(GM/R) \sim 10^{-1} c^2$, while that released by very rapid (timescale < 10 s) thermonuclear fusion of any nuclear fuel will be $\lesssim 10^{-3} c^2$ (refs 76, 77). Hence, this type of model predicts that the ratio, α , of time averaged persistent accretion-driven X-ray luminosity to time averaged burst luminosity should exceed $\sim 10^2$. This criterion is apparently met by some burst sources with rather bright steady X-ray counterparts (Table 1 and refs 5, 6). However, it apparently fails in the case of the rapid burster, for which $\alpha \lesssim 2$ during times of burst activity²¹. It also seems to fail for MXB1659-29, whose observational properties are similar to those of most other burst sources (unlike the rapid burster) but which has recently been found to have $\alpha < 25$ during burst-active times (unpublished SAS-3 results). Thus, it seems that this mechanism can account for no more than a subset of the presently known burst sources. In any event, a complete evaluation of the physics of thermonuclear flashes in neutron-star surface layers has yet to be carried out.

If the burst sources, are in fact, compact objects of moderate mass, then they may be accreting matter from close binary stellar companions. In this respect, they may be very similar to other binary X-ray sources, which are also widely believed to consist of compact objects that are undergoing accretion from relatively normal companion stars (see ref. 78 and references therein). If the burst sources are in such systems, one might expect to find periodic temporal variability in the associated steady X-ray emission or in the optical candidates. As noted above, however, no such periodic variability has been reported to date.

The blackbody spectra reported for three burst sources (see section on burst spectra and refs 17-19) are the only direct indication to date that burst sources are neutron stars or black holes of stellar mass.

The mechanism which produces X-ray bursts is thus not yet known, but the most promising mechanisms are based on the

accretion of matter onto a compact object of Stellar mass.

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Note added in proof: Recently, Hoffman, Marshall and Lewin⁸⁰ discovered that the rapid burster occasionally produces 'special' bursts which, unlike the rapidly repetitive bursts, show the typical characteristics of bursts from other sources (similar time scale of burst intervals and similar spectral evolution during burst decay). They suggest in a recent preprint that rapidly repetitive bursts may be a replacement of the persistent emission observed from many other burst sources and that in this special case the persistent emission manifests itself as bursts. If this were so, it opens the interesting possibility that the rapidly repetitive bursts may be the result of instabilities in the accretion flow (gravitational potential energy) but that the 'special' bursts from the rapid burster and the X-ray bursts from all other sources are the result of thermonuclear flashes.

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articles

Oxygen isotope and palaeomagnetic evidence for early Northern Hemisphere glaciation

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Oxygen isotope and palaeomagnetic analysis of the lower half of LDGO piston core V28-179 shows that glacial-interglacial fluctuations have characterised Earth's climate for the past 3.2 Myr, before which there was a period of stable 'interglacial' or 'preglacial' climate. The scale of glaciations increased about 2.5 Myr ago.

THE stratigraphically longest detailed oxygen isotopic records from the oceans extend to sediments about 2.1 Myr old^{1,2} and indicate Pleistocene-like glacial events well below the horizon stratigraphically equivalent to the base of the Quaternary as it is defined in Italy³. Here we extend the isotopic record and identify the onset of these quasi-cyclic glacial-interglacial fluctuations. For this we required a core of sediment that accumulated somewhat more slowly than in the area of our previous studies.

Cores from the Equatorial Pacific were described by Hays *et al.*⁴, who showed that north of the belt of maximum biological productivity along the equator it is possible to take piston cores containing a record extending well into the Pliocene. They discussed the lithostratigraphy and magnetostratigraphy of several cores, and the biostratigraphic record for foraminifera, diatoms and radiolaria (expanded in refs 6 and 7) while Gartner⁵ presented the nannofossil stratigraphy. Since then several cruises have recovered cores from the same general area. Of these we selected V28-179 (collected by N.D.O.), for isotope analysis because it contained the thickest accumulation of sediment for the Gauss magnetic epoch.

Core V28-179 was taken at 4° 37'N, 139° 36'W in 4,509 m water depth. The core comprises alternating layers of foraminiferal chalk ooze, marl ooze and marl with some dominantly diatomaceous layers. Most of the core is intensely burrowed, and many of the plugs of sediment washed for analysis could be seen to contain components of slightly different colour mixed

Fig. 1 Magnetic record for core V28-179, 150 Oe and interpretation in terms of the standard palaeomagnetic polarity scale.

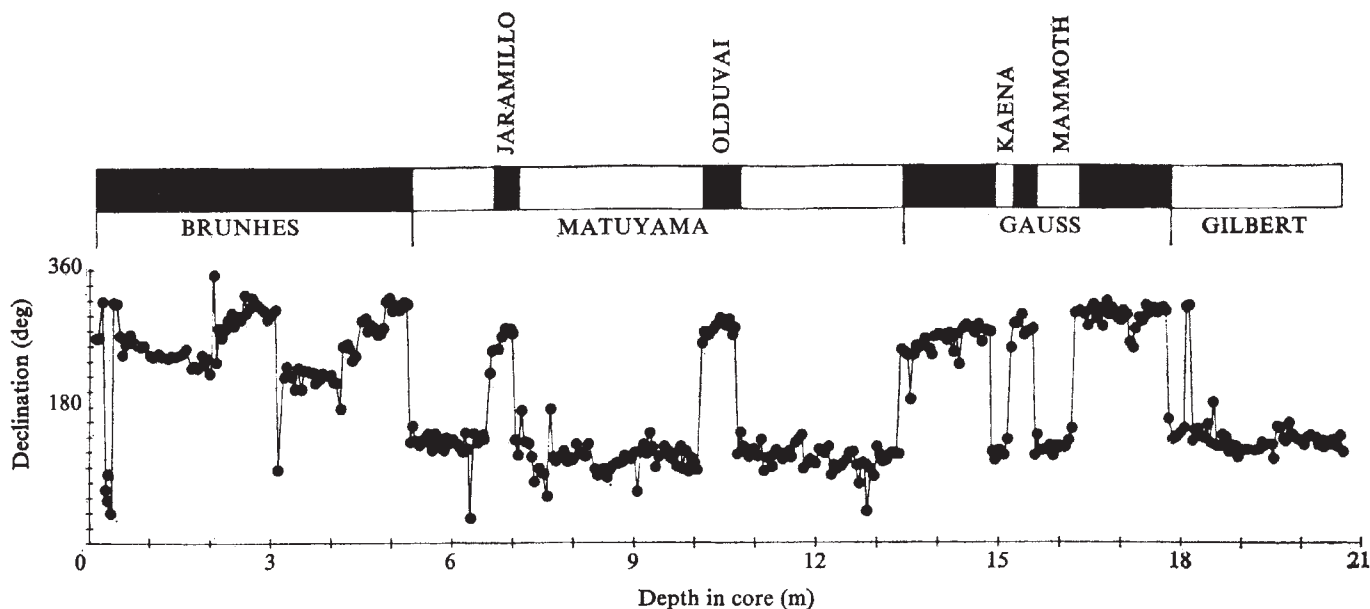


Table 1 Magnetic boundaries V28-179

Brunhes-Matuyama	527 ± 2.5 cm
Matuyama-Jaramillo	657 ± 4 cm
Jaramillo-Matuyama	700 ± 2.5 cm
Matuyama-Olduvai	1,007 ± 2.5 cm
Olduvai-Matuyama	1,068 ± 3 cm
Matuyama-Gauss	1,358 ± 2.5 cm
Gauss-Kaena	1,488 ± 2 cm
Kaena-Gauss	1,517 ± 2.5 cm
Gauss-Mammoth	1,558 ± 2 cm
Mammoth-Gauss	1,622 ± 2.5 cm
Gauss-Gilbert	1,779 ± 2.5 cm
Unnamed short interval (1,808-1,817 ± 2.5 cm)	

together by burrowing. This strongly suggested that the depth through which burrowing took place was frequently greater than the typical thickness of a single layer deposited under uniform environmental conditions. We assume, following evidence already available⁹, that the chief source of variability in the sediment derives from climatically controlled variations in the proportion of carbonate remaining in the sediment. As these variations occurred on a depth scale that was smaller than the depth of plainly imperfect homogenisation by burrowing organisms, it shows that we are limited to an investigation of the general character of the ocean oxygen isotopic record within the good time-framework provided by the palaeomagnetic record of the core.

The record of changing ocean oxygen isotopic composition in the Late Pleistocene has been investigated in some detail in cores with accumulation rates up to about 8 cm kyr⁻¹ (ref. 10), and it has been shown that some cores with accumulation rates of over 3 cm kyr⁻¹ preserve a sufficiently good record to allow the identification of the effect of the changing tilt of the Earth's orbit (period 40 kyr) and of precession of the equinoxes (period about 23 kyr)¹¹. To investigate the Lower Pleistocene in a piston core, it is necessary to work in an area of rather lower sediment accumulation. We have already discussed¹ the loss of information which characteristically occurs under these conditions. Even if the mixing in the sediment of core V28-179 were homogeneous, climatic fluctuations with a 40 kyr period would have a wavelength in the sediment of only about 20 cm and would be scarcely detectable. Since the mixing is clearly not homogeneous, and since several samples proved too low in carbonate for an analysis to be made at all, it is likely that the detailed character of the climatic record is inaccessible. What we can do is to identify the date of inception of the isotopic fluctuations which have characterised at least the whole of the Pleistocene¹. Although there are other types of evidence which indicate periods of climatic deterioration, there is as yet no information pertaining to the date of the earliest accumulation in the Northern Hemisphere of continental ice sheets that would have been of sufficient scale to produce a detectable effect on the oxygen isotopic composition of the oceans¹².

The core was sampled for magnetic measurement at 5 cm intervals along its length. The measurements were performed on a slow speed fluxgate magnetometer of the type described by Molyneux¹³. In order to ascertain the proper alternating field to use for blanket demagnetisation and to determine the magnetic stability of the core 10 samples spaced throughout the core were progressively demagnetised in peak alternating fields of up to 250 Oe. The magnetic stability was found to vary throughout the length of the core with values of the median destructive field ranging from 25 to 225 Oe. Natural remanent magnetic intensities ranged from 0.2 to 20 × 10⁻⁶ e.m.u. cm⁻³. A field of 150 Oe peak value was selected for blanket demagnetisation. The resulting plot of declination change against depth is shown in Fig. 1 plotted relative to an arbitrary fiducial mark scribed on the ship during the extrusion process¹⁴. The correlation of the resulting magnetic pattern to the standard time scale is easily done since the reversals are clearly delineated. This correlation has been confirmed by foraminiferal studies¹⁵

using the foraminiferal zonation previously determined for equatorial Pacific sediments^{4,6,7}.

An important fact is that the pattern of coiling changes in *Pulleniatina* and in *Globorotalia tumida* shows that the lowest reversely magnetised section of sediment below about 18m can only represent the interval from the Gilbert-Gauss boundary to a horizon somewhat later than the Cochiti event; the bottom of the core probably has an age between 3.5 and 3.6 Myr (ref. 15).

Table 2 Oxygen and carbon isotope data for *Globocassidulina subglobosa* from core V28-179

depth δ(cm)	¹⁸ O	¹³ C
1,011	+4.30	-1.44
1,031	+3.79	-1.35
1,051	+4.03	-1.24
1,061	+3.52	-1.10
1,070	+3.67	-0.73
1,080	+4.21	-1.05
1,090	+3.89	-0.87
1,129	+3.86	-0.85
1,140	+4.14	-1.14
1,150	+3.72	-0.83
1,170	+3.48	-0.67
1,180	+4.31	-1.11
1,190	+3.77	-0.62
1,220	+3.79	-1.24
1,241	+3.80	-0.85
1,271	+4.15	-1.29
1,280	+3.46	-0.96
1,291	+4.02	-1.28
1,310	+4.30	-1.61
1,321	+3.65	-0.98
1,341	+3.98	-1.40
1,351	+3.99	-0.87
1,361	+3.41	-0.54
1,370	+4.05	-1.10
1,381	+3.94	-1.08
1,391	+3.29	-0.65
1,420	+3.92	-0.69
1,442	+3.55	-0.85
1,450	+3.77	-0.92
1,480	+4.02	-1.04
1,510	+3.42	-0.67
1,520	+3.87	-0.92
1,530	+3.36	-0.60
1,580	+3.93	-0.96
1,590	+3.80	-1.18
1,600	+3.92	-1.01
1,610	+3.35	-0.92
1,620	+3.76	-0.61
1,631	+3.69	-0.88
1,660	+3.66	-0.94
1,670	+3.60	-0.74
1,680	+3.52	-0.68
1,690	+3.13	-0.78
1,700	+3.50	-0.88
1,711	+3.16	-1.04
1,721	+3.19	-0.57
1,731	+3.19	-0.71
1,741	+3.37	-0.53
1,771	+3.13	-0.70
1,780	+3.43	-0.42
1,791	+3.53	-0.38
1,800	+3.14	-0.33
1,810	+3.56	-0.88
1,820	+3.19	-0.47
1,830	+3.28	-0.55
1,840	+3.30	-0.73
1,870	+3.25	-0.48
1,890	+3.20	-0.15
1,900	+3.41	-0.91
1,911	+3.29	-0.82
1,920	+3.20	-0.51
1,950	+3.40	-0.39
1,940	+3.24	-0.83
1,961	+3.36	-0.96
1,990	+3.40	-0.79
2,030	+3.30	-0.67
2,040	+3.51	-0.62
2,051	+3.45	-0.76
2,060	+3.14	-0.96

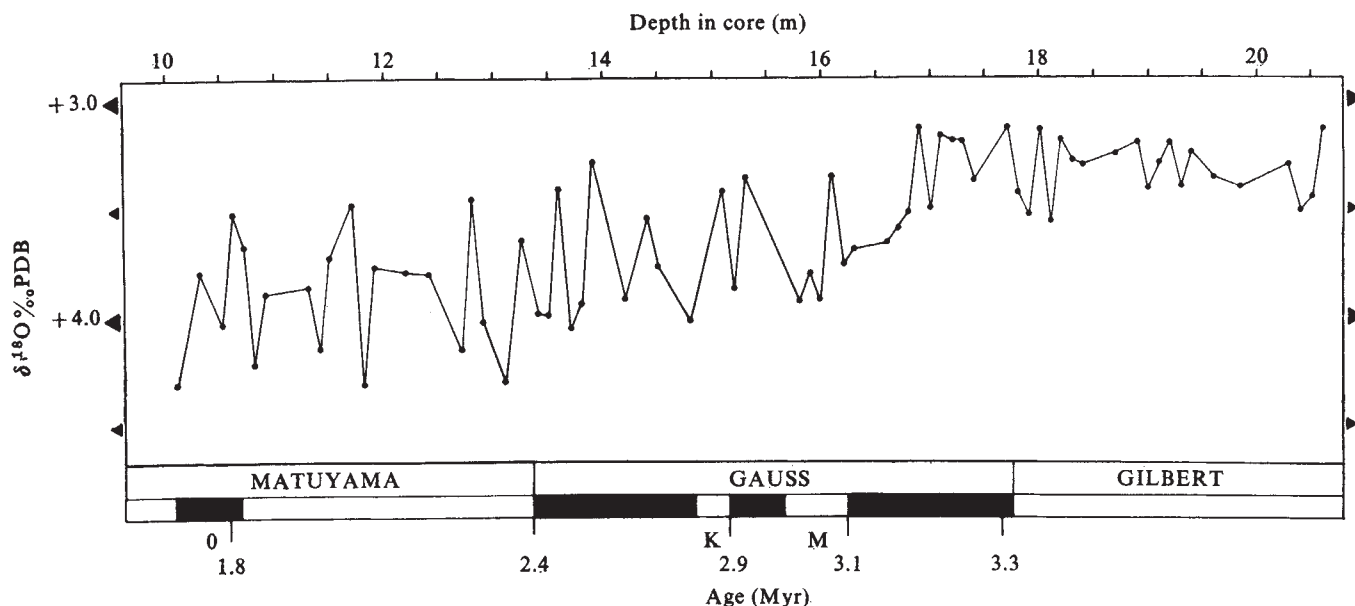


Fig. 2 Oxygen isotopic composition of *Globocassidulina subglobosa* in core V28-179 from 10m to 21m. *G. subglobosa* living on the sea floor at the coring site today would have an ^{18}O content of about +3.50‰. Magnetic events are indicated by O (Olduvai), K (Kaena) and M (Mammoth). Ages indicated are based on current estimates of the chronology for the magnetic record²⁶.

The placement within the core of the major palaeomagnetic transitions are given in Table 1. The only departure of the magnetic stratigraphy from the standard magnetic time scale is the presence of a short normally magnetised interval at 1,800 cm, below the Gauss normal magnetic epoch. It is possible that this represents a short reversal of the magnetic field since the core does not show any apparent physical disturbance at this level.

Oxygen isotope analyses were made at 10 cm intervals in the samples previously used for magnetic studies. The samples were dispersed in distilled water in a mechanical shaker, and after brief ultrasonic treatment, they were sieved on a 125 μm mesh and dried in a cool oven on the sieves. Foraminifera for isotopic analysis were taken from this fraction further cleaned ultrasonically and purified by roasting *in vacuo* at 400 °C for 30 min. Carbon dioxide was released from the carbonate by the action of 100% orthophosphoric acid at 50 °C and analysed in a VGMicromass 602C mass spectrometer; analyses are reported to the PDB standard on the basis of multiple analyses of circulating standard carbonates.

The preservation of foraminifera is very poor at the water depth at which core V28-179 was taken (4,509 m). It was, therefore, generally not possible to obtain for analysis *Globigerinoides sacculifer*, the species used in our previous studies^{1,8}. Instead, we chose to analyse benthonic foraminifera, which are less susceptible to dissolution. As many species of benthonic foraminifera do not deposit their carbonate in isotopic equilibrium with the sea water they inhabit¹⁶, it would clearly be desirable to analyse a single species throughout. *Uvigerina*, known to deposit carbonate in isotopic equilibrium, is extremely rare, but in the majority of samples we were able to extract sufficient specimens of *Globocassidulina subglobosa* for analysis.

To calibrate *G. subglobosa* for possible departure from isotopic equilibrium, we analysed the top part of core V19-28 from which we obtained excellent isotopic data for *Uvigerina*^{10,17}. Our measurements show that *G. subglobosa* may be even closer to isotopic equilibrium than *U. senticosus*, the species analysed in core V19-28. Comparisons of the oxygen isotopic composition of *G. subglobosa* and of *U. spinulosa* in Oligocene samples confirms the suitability of this species for oxygen isotope work^{18,19}. The bottom temperature at the site of core V19-28 is about 1.8 °C; at the site of V28-179 it is about 1.1 °C. The mean ^{18}O content of *Globocassidulina subglobosa* in the upper part of core V19-28 is +3.30‰ (five analyses); using the relationship $T = 16.9 - 4.4\delta + 0.10\delta^2$ (refs 20, 21) enables us to estimate that the temperature difference between

the two sites should be equivalent to an isotopic difference of about 0.2 ‰, so that the expected value at the V28-179 site is about +3.50 ‰. Since the sediment accumulation rate at the coring site is only about 0.6 cm kyr⁻¹, the core top would be likely to contain a mixture of glacial and recent specimens and would not be useful for calibration purposes.

Analytical results for *G. subglobosa* are given in Table 2, and are plotted in Fig. 2. The record may best be described as a caricature of the true course of events, in that it is certainly not possible to derive an accurate record of events in such gradually accumulating sediment. It is, however, quite clear that the uppermost 17 m of the core are predominantly glacial in character; below 17 m, isotopic values are consistently close to the present-day value. Taking this lower section first, we may conclude that between about 3.5 Myr and 3.2 Myr ago no big ice sheets accumulated in the Northern Hemisphere. Isotopically, the ocean was in an interglacial state, with more or less constant isotopic composition. The standard deviation among the measurements for this part of the core is 0.13 ‰. If this were entirely due to real variability, it would represent glacial events of a magnitude up to the equivalent of a 26 m range in eustatic sea-level fluctuation⁸. The variations are on so small a scale that if they derive from real glacial events, then they could be caused by changes in the Antarctic ice sheet, or by small Northern Hemisphere glaciations. It is conceivable, however, that this variability stems entirely from analytical error. More accurate measurements in a section with more rapid sediment accumulation will be needed to investigate possible small-scale glacial events earlier than 3.2 Myr ago.

Just below the Mammoth event at 1,680 m we see a positive excursion in isotopic composition that is well above possible analytical uncertainty and is of such a magnitude (0.4 ‰) as to represent about a 40 m sea-level equivalent in stored ice (assuming its isotopic composition was about -35 ‰). This date is in substantial agreement with the inception of ice-rafting observed in sediments from site 116 in Leg 12 of the Deep-Sea Drilling Project²² and with evidence of glaciation in Iceland²³ and with ref. 12, but the present data give for the first time the scale of fluctuations which have continued ever since.

At the base of the Matuyama (sample at 1,310 cm) a glacial isotopic excursion of about 1.0 ‰ is observed. This is a characteristic extreme value over the lower Matuyama section. In deep-Pacific cores with a high accumulation rate the maximum difference between glacial and interglacial isotopic values in

the late Pleistocene is about 1.60 per mil (ref. 10). Thus we have clear evidence that glaciations of a magnitude of at least two-thirds that of the late Pleistocene glacial maxima were occurring in the time interval from 2.5 to 1.8 Myr ago. This was probably the scale of glaciation throughout the Lower Pleistocene¹. Evidently a major change in the character of glaciations occurred at about 2.5 Myr ago. The substantial carbon isotopic event at that point in the record may represent a large drop in the continental biomass²⁴ and may have been associated with significant floral extinctions under severe environmental pressure²⁵.

Few localities are likely to preserve a sea-level record for the past 3 Myr. But, it may be deduced from Fig. 2 that the last lengthy period of stable sea level and low ice volume terminated about 3.2 Myr ago, so that it is likely that a major constructional coral terrace feature is present in some areas dating from this time; marine highstands in the remaining Pliocene, like those of the Pleistocene, were probably brief. On the basis of this analysis one might advance the hypothesis that before 3.2 Myr ago, continental environments were relatively stable over a long period of time; since then the Earth has been subjected to the stress of continually fluctuating climate, with pleasant climates like our own occurring infrequently and lasting only about 10,000 years.

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Immunological specificity and memory in a scleractinian coral

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Tissue transplantation immunity with a specific memory component is demonstrated in populations of Montipora. This highly discriminating immunoreactivity derives from extensive allogeneic polymorphism of histocompatibility (H) markers. An H system of immunorecognition is postulated to have originated in multicellular invertebrates probably beginning with coelenterates.

POSSESSION of a specific immune system, including immunorecognition leading to selectively inducible responses with a memory component, has long been considered unique to vertebrates. Although invertebrate defence reactions were thought to lack sharp specificity, we now know that metazoans ranging from coelenterates to protochordates possess a well-developed capacity for recognition of foreign tissue followed by antagonistic reactions. Among higher invertebrates, immunorecognition at the allogeneic level has been repeatedly described in annelid worms^{1,2}, echinoderms^{3,4}, and tunicates^{5,6}. Convincing evidence of allogeneic incompatibility in coelenterates has been reported from controlled experiments with colonial hydroids⁷, gorgonians⁸, and anthozoans, including sea anemones^{9,10} and corals^{11,12}. The situation in sponges, the most primitive of multicellular animals, is less clear. Certain species exhibit allogeneic recognition yielding non-fusion of separate clones after an initial period of adhesion, but cytotoxic reactions have not been reported¹³.

Continuing scepticism concerning the existence of immunocompetence in invertebrates^{14,15} focuses on ques-

tions of specificity, memory and the molecular basis of cytotoxic reactivity. A specific alloimmune memory component to transplantation immunity among higher invertebrates has already been documented in annelids⁶ and in echinoderms⁴. In living corals, specific reactivity is demonstrated by consistent compatibility of intracolony or syngeneic transplants, while intercolony allografts and interspecific xenografts are invariably incompatible. These distinct reactions, characteristic of many scleractinian corals, are both naturally-occurring on tropical reefs and reproducible in laboratory experiments^{11,17}. Quasi-immune surveillance in corals, with manifestations ranging from mild to severe, is evidenced by contact avoidance reactions, allogeneic contact incompatibility, chronic xenogeneic incompatibility and acute interspecific cytotoxicity. Positive recognition of foreignness in the form of non-fusion in areas of tissue contact precedes cytotoxic reactions which usually require a longer period of sensitisation. Examination of allogeneic graft reactions in *Montipora verrucosa*, a foliaceous reef-building coral abundant in the Hawaiian Islands, surprisingly suggested a specific immunorecognition system with a memory component of at least short-term duration¹². We now present an extensively controlled study of the essential immunocompetence of this coelenterate as a function of polymorphism of histocompatibility (H) markers, specificity and memory.

Experimental tissue grafts

M. verrucosa is most attractive for tissue grafting because of its flat configuration and rapid healing. Many techniques for placing pieces of coral in firm, soft-tissue contact have

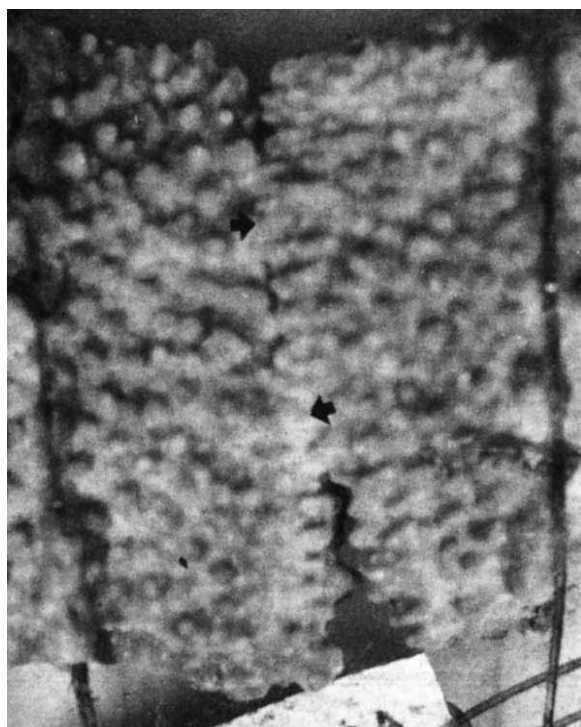


Fig. 1 Intracolony isografts of *M. verrucosa* showing compatible fusion at interface (arrows) at 30 d after grafting. Technique of pair grafting on plexiglas base using monofilament nylon tie-downs is also illustrated. Approximately $\times 1$.

been tried. Notched plexiglas rectangles about $23 \times 12 \times 1$ cm and high-test tie-downs of monofilament nylon proved adequate for grafting paired *Montipora* pieces ranging in dimensions from 9–72 cm² (Fig. 1). Heavy bone shears were used to cut pieces to desired size, but only uncut edges were joined as graft interfaces. Grafts were established by tying together laterally two pieces of living coral with 50 pound test monofilament nylon on plexiglas plates, using small lead crimps to bind the nylon firmly. All coral was maintained in a rapidly running and unfiltered seawater system at 24–26 °C; in these conditions at the University of Hawaii Facility at Leiliwi Bay, *Montipora* thrived for for many months. Coral pieces were kept immersed in seawater in large trays while grafting and scoring.

Tissue reactions at transplant interfaces were scored under a large platform stereomicroscope, usually at $45\times$ magnification. Useful indicators of graft viability versus rejection were (1) allogeneic contact avoidance/inhibition evident by failure of soft tissues to fuse or grow across small gaps; (2) persistence or disappearance of pigmented zooxanthellae in coenenchyme at contact points; (3) hyperplasia/blanching/death of soft tissue in contact zones. Cytotoxic reactions of allogeneic incompatibility may be either unilateral or bilateral when first observed, but nearly all became bilateral in *Montipora*. For purposes of quantitating cytotoxic reaction times of allografts, zones of soft tissue death of 1 mm or more from either side of the interface were scored as definitive. This extent of tissue death is unequivocal when measured under the stereomicroscope (Fig. 2) and allowed different investigators to score the same grafts on successive days with objectivity. Initial allocytotoxic reactions of 0.5 mm or less may take 2–3 weeks to become definitive in occasional slow reactors.

Technical trauma sometimes causes slight tissue destruction (<0.5 mm) that is evident within 24 h of establishing grafts, but which heals within 1 week after grafting. Unless otherwise stated, all isografts, first-set and repeat-set allo-

grafts in the present experiments were 12–16 cm² in area. Soft tissue to a depth of 1–2 mm overlies the hard calcareous skeleton. Other technical considerations have been described elsewhere^{12,17}. Three widely separate populations of *M. verrucosa* from the island of Hawaii were used. Specific first- and second-set allografts involved comparison of Kapoho and Onekahakaha populations, and unrelated third-party allografts were from the City of Refuge reef. Time–percentage effect curves, reaction time ratios and slope function ratios were determined by nomographical methods¹⁸.

Evidence of alloimmune specificity and memory

Control intracolony isografts were always compatible as shown by soft tissue fusion in all contact areas as early as 3–5 d after grafting. Neither blanching nor tissue hyperplasia accompanied this fusion in some 18 pairs tested. By 12–15 d, coenenchyme continuity with confluent zooxanthellae was observed throughout contact zones. Calcification was already well underway at interfaces, but gaps of 3 mm or more remained open. Smaller gaps disappeared as a result of bilateral outgrowth. By 24–30 d, complete reconstitution indistinguishable from normal ungrafted coral was evident (Fig. 1). This compatible fusion then persisted indefinitely.

Rejection of initial or first-set allografts was preceded by a lag or sensitisation period of 2–3 weeks during which time intimate soft tissue contact was established. Antagonistic reactions were then revealed by progressive blanching and loss of soft tissue in the immediate contact area. In a series of 32 test pairs, primary allografts yielded a median reaction time (MRT) of 22.0 (19.2–25.3) d. Secondary allografts, established by resetting the same coral pairs at new interfaces distant from the original contact area, showed accelerated and intensified cytotoxic reactions with an MRT of 11.6 (9.4–14.4) d. This MRT difference with an interval of 40 d between first-set and second-set grafting (Table 1)

Fig. 2 Intercolony allografts of *Montipora verrucosa* showing bilateral cytotoxic incompatibility restricted to immediate contact zone. Note blanching and soft tissue death at interface (arrows). Approximately $\times 1$.

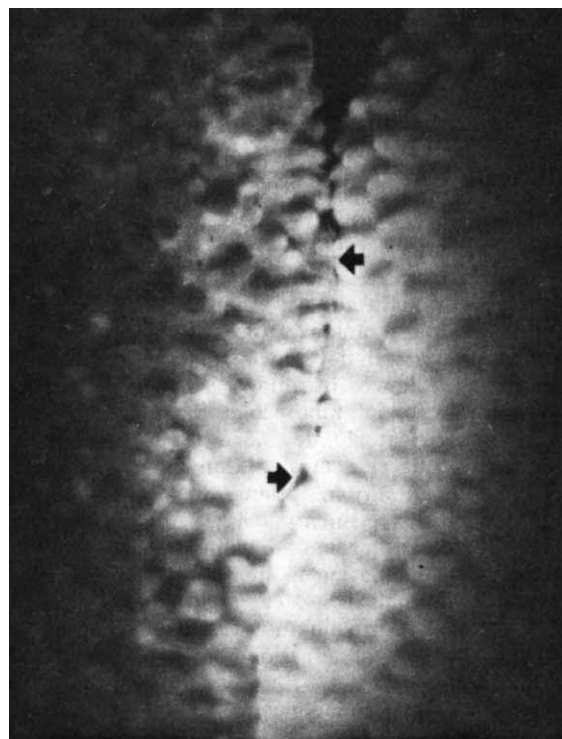


Table 1 Evidence of specificity and memory in allograft reaction times of *Montipora verrucosa*

Allograft expt	No. coral pairs scored	Median reaction times (MRT) (d)*	Range of individual cytotoxic reaction times (d)	Interval between 1° and 2° grafting (d)§	Comment
First-set	32	22.0 (19.2–25.3) (9.2)	16–40	—	
Second-set	18	11.6 (9.4–14.4) (5.7)	9–23	40	MRT difference between 1° and 2° grafts highly significant†
Unrelated third-party Biphaseic‡	21				
(1) Slow reactions	8	22.9 (18.4–28.5) (7.4)	23–35	41	MRT not significantly different from 1°; specific induced immunity not evident
(2) Rapid reactions	13	13.2 (9.7–18.0) (8.2)	8–17	41	MRT not significantly different from 2°; induced immunity is evident

*Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRT's.

†Slopes of curves for first- and second-set reactions were parallel within experimental error, suggesting that same qualitative events occurred, but at different rates.

‡MRT difference between slow and fast reactors is significant at 95% confidence level. Note also that these two groups were qualitatively distinctive: slow reactions were concurrently bilateral, whereas rapid reactions were unidirectional as expected with one-way pre-immunisation.

§1°, First-set and 2°, second-set grafting.

was significantly suggestive of immune memory. Intensified second-set reactivity was evidenced by accentuated early hyperplasia and secretion of mucus at interfaces.

To test for specificity, 21 pairs of third-party grafts from a geographically distant City of Refuge population were performed with first-set rejectors from either Kapoho or Onekahakaha sources. These third-party grafts yielded a broad range of cytotoxic reaction times reflecting a biphasic distribution of accelerated and non-accelerated responses (Table 1). The rapid reactions (61%) were conspicuously unilateral, restricted to the third-party grafts as expected for unidirectional pre-immunisation. Conversely, the remaining slow reactions, not significantly different from first-set reactions, were concurrently bilateral and typical of primary reactions (Fig. 3). In other words, third-party grafts were distinguishable qualitatively on the basis of unilateral versus bilateral reactivity as well as quantitatively on the basis of cytotoxic reaction times. These striking results reveal both specificity in transplantation alloimmunity and extensive antigenic polymorphism among genetically separate *M. verrucosa*. The impressive polymorphism of H markers in this species is also confirmed by the incompatibility of all of some 280 intercolony graft combinations tested thus far within and between Onekahakaha, Kapoho and City of Refuge populations.

To what extent does allograft size influence direction, rate of appearance or degree of severity of cytotoxic reactions? Allografting in pairs at tissue mass ratios of 2:1, 4:1, and 8:1 was accomplished with the smaller piece the same size (9 cm²) in three experiments (Table 2). Within this range, graft dosage had little or no effect on the direction or timing of allograft reactivity. Initial cytotoxic reactions were scored with equal frequency on small or large grafts. All allograft reactions became bilateral in the same manner as control grafts of equal-size colonies. Very small pieces of *Montipora* seem more vulnerable to stress and disease in suboptimal conditions.

Induction and duration of alloimmune memory

The occurrence of specific short-term memory was demonstrated by the second-set and third-party reactions evoked when appropriate repeat grafts were placed soon after initial graft pairs had responded incompatibly (Table 1). The questions arise: how long does such positive memory persist and how long does it take to induce such memory? The duration of alloimmune memory was tested

by second-set grafting pairs of corals at new interfaces at 2, 4, 8 and 16 weeks after separation from primary allografting. A 28-d period of primary contact was chosen to assure full sensitisation, since cytotoxic reactions were then underway in all graft pairs. Strong alloimmune memory was present in the second-set group grafted two weeks after first-set separation as shown by an early MRT of 10.7 (8.6–13.4) d (Table 3). This heightened reactivity had faded considerably in *Montipora* test-grafted after 4 weeks. At 8 weeks after first-set separation, specific memory was no longer detectable as evidenced by a MRT of 21.8 (16.1–29.4) d, quite similar to that of control first-set grafts. No further change was demonstrable after 16 weeks.

Possible early induction of memory was tested by disjoining initial allografts after 1, 2, 4 or 8 d of contact and regrafting the same pairs at new interfaces 3 d later. Eight coral pairs regrafted after only 1 d of allogeneic

Fig. 3 Logarithmic probability plots of first-set (●), second-set (×) and third-party (○) allograft reaction times of *M. verrucosa*. MRT difference between 1° and 2° grafts (22.0 and 11.6 d respectively) was highly significant; parallel slopes suggest same cytotoxic events occurred, but at different rates. Unrelated 3° allografts in lieu of specific 2° grafts displayed biphasic distribution of primary-type (3₁) and secondary-type (3₂) rejections.

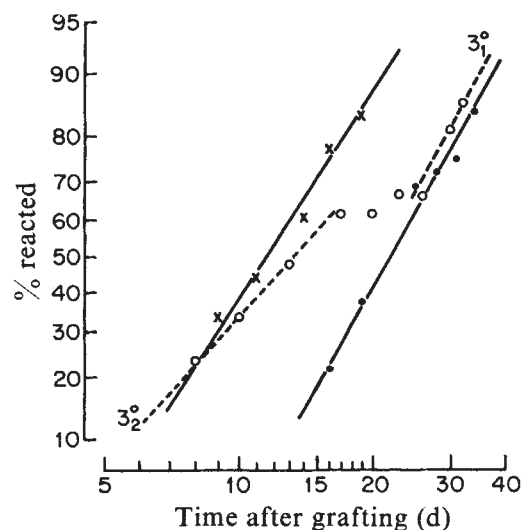


Table 2 Tests for influence of allograft size on cytotoxic reaction times in *M. verrucosa*

Allograft sizes*	No. coral pairs tested	Median reaction times (MRT) (d)†	Range of individual cytotoxic reaction times (d)
(1) 18 × 9 cm ² (2 : 1)	8	18.2 (13.9–23.9) (7.3)	14–39
(2) 36 × 9 cm ² (4 : 1)	8	20.9 (16.3–26.7) (7.8)	11–47
(3) 72 × 9 cm ² (8 : 1)	8	24.4 (19.1–31.3) (9.1)	11–47

Graft dosage or size had little or no effect on the direction or timing of allograft reactivity.

*Tissue mass ratios shown in parenthesis. Because the smaller piece was the same size (9 cm²) in all three experiments, the amount of allogeneic tissue in interfacial contact was also the same.

†Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRTs. Initial cytotoxic reactions were discernible with equal frequency on small or large grafts; all allograft reactions became bilateral in the same manner as control grafts of equal-size pairs.

contact yielded a MRT of 18.6 (15.5–21.6) d with a range of individual cytotoxic reaction times of 17–45 d. Similar results were obtained in three additional experiments with short-term presensitisation attempted for 2, 4 or 8 d. Individual cytotoxic reaction times ranged from 9–35 d, but only one test-allograft in each of these experiments exhibited an early reaction suggestive of induced immunity. Reaction time ratio tests showed that none of the four MRT's obtained was significantly different from the control first-set value of 22.0 d. Thus little or no pre-immunisation was conferred by short-term allogeneic contact in marked contrast to the potent immunity that resulted from contact for 28 d or longer. These results suggest that systemic immunisation requires longer than 8 d of cell-surface contact. Alternatively, any early immune memory induced seems to fade rapidly.

Implications of immunoreactivity in lower invertebrates

In the light of earlier studies of advanced invertebrates, we were not surprised to find highly discriminating transplantation specificity in corals coupled to extensive polymorphism of allogeneic H markers probably abundant on cell surfaces. The decisive experiments were observable in nature, whereas our challenge was to quantitate the reactions in controlled laboratory conditions. The finding of specific alloimmune memory in coelenterates was

unexpected, however, even though we realised that memory itself might well be an evolving characteristic in multicellular animals¹⁹. This supposition is now strengthened by the repeated demonstration of short-term memory in *Montipora* in this study. Further investigation of conditions that might favour long-term memory is desirable, because the absence of this characteristic could be a major distinguishing feature of lower metazoans.

The histopathological changes accompanying allocytotoxicity in *Montipora* have proved more difficult to interpret because the diversity of leukocytes or inflammatory cells associated with allograft reactions in higher animals is not evident in coelenterates. Moreover, appropriate techniques for fixation, decalcification and staining of coral tissues are not fully developed. Although we have many histological sections of *Montipora* allograft reaction zones at successive stages of incompatibility, cytotoxicity is obviously reflected only in tissue disruption, localised loss of cells in the immediate contact zone, and excessive mucus secretion. No lymphocytic-or phagocytic-type cells, as found to infiltrate allografts in more advanced invertebrates^{3–5}, have been detected thus far in our coral grafts. Although coelenterates including corals are endowed with leukocyte-type cells called amoebocytes²⁰, their participation in allograft rejection reactions remains conjectural.

Elsewhere a two-component system of (1) specific H marker immunorecognition leading to (2) nonspecific cytotoxic reactions mediated by non-antibody proteins has been suggested as the essential basis for immunocompetence in multicellular invertebrates²¹. The specific induction phase is apparently contingent on polymorphic H receptors leading to activation or triggering of a nonspecific effector phase served by macromolecules with broadly toxic properties. Although specific cell-surface immunorecognition is characteristic of the solitary coral *Fungia*, the non-dialysable, xenocytotoxic molecule(s) secreted in response are nonspecifically reactive with diverse target cells^{11,17}. This early but efficient form of immunoreactivity seems to have been retained during progressive evolution of immunocytes and diversification of functions.

The immunoglobulin (Ig) system of antibody-mediated immunity first appears at the level of chordates or fishes as an addition to the postulated H system of cell-mediated immunity already well-developed among invertebrates. Cooperation among lymphocytes and macrophages apparently led to progressive integration of the H and Ig systems eventuating in the immunoregulatory network characteristic of higher vertebrates^{21,22}. The heterogeneity of classes of thymus-derived T lymphocytes serving numerous functions among vertebrates, including transplantation immunity, may involve distinctive H and Ig receptors²³. Possibly the Ig molecules of vertebrates evolved from structurally similar H molecules^{24,25} to provide physiologically complex animals with two immunorecognition systems.

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Table 3 Duration of alloimmune memory in *M. verrucosa* after separation from primary allografting at 4 weeks

Time of second-set grafting (weeks after 1 st separation)	No. coral pairs tested	Median reaction times (MRT) (d)*	Range of individual cytotoxic reaction times (d)
2	7	10.7 (8.6–13.4) (3.4)	9–17
4	8	15.3 (11.4–20.6) (6.8)	10–34
8	7	21.8 (16.1–29.4) (9.3)	12–38
16	7	20.1 (14.6–27.8) (9.1)	12–35

Strong alloimmune memory was present at 2 weeks, but this memory faded considerably by 4 weeks and was completely gone by 8 weeks after primary separation.

*Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRTs.

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Spatial configuration of mRNA 5'-terminus

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Nuclear magnetic resonance investigation has revealed that the 5'-terminus m⁷G^{5'}ppp^{5'}Am of mRNA displays a spatial configuration in which the bases form stacked arrays. Details of the conformation as derived from coupling constants, shift trends and ring current considerations are discussed.

THE 5'-terminal regions of a wide variety of mRNAs of eukaryotes contain an unusual sequence of nucleotides in which the terminal is 7-methyl-guanosine¹⁻¹¹ (Fig. 1). Several studies¹⁰⁻¹⁵ have shown that this sequence for the 5' termini of mRNAs is obligatory to carry out *in vitro* translation with fidelity.

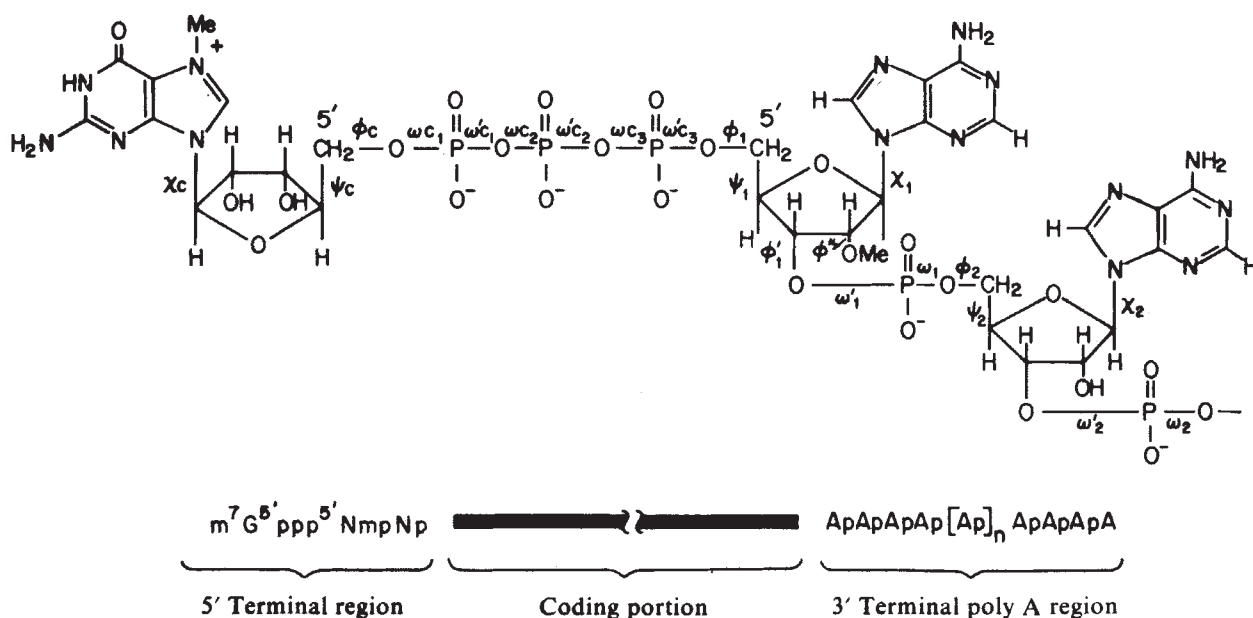
To understand the structure-function relationships we have explored whether the uncommon constitution of the 5' termini endows any unique stereochemical features to mRNAs. The conformational properties of the 5' termini were investigated by studying the solution conformations of: (1), 7-methylated guanosine derivatives compared with the corresponding non-methylated analogues; (2), 2'-O-methylated nucleosides and the corresponding 5'-nucleotides compared with the non-methylated analogues; and (3), the dimer m⁷G^{5'}ppp^{5'}Am.

All materials used were commercial preparations. ¹H nuclear magnetic resonance (NMR) spectra were recorded at 270 MHz in the Fourier transform mode using systems described elsewhere¹⁶⁻¹⁸. All NMR spectra were analysed using a UNIVAC 1110 computer and LACON III. Figure 2 illustrates the observed and computer-simulated spectra of m⁷G^{5'}ppp^{5'}Am at two temperatures. The coupling constants data derived for the various monomers and the dimer were translated into conformational parameters using equations developed by Lee and Sarma¹⁹ and Lee *et al.*¹⁸ and the data are summarised in Table 1.

Conformational properties of 7-methylated monomers

In general the ribose ring of common nucleosides and nucleotides at the examined pH and temperature exist as ²E $\rightleftharpoons$ ³E equilibrium mixture with a bias of $\approx 60\%$ for ²E conformer (Table 1). Inspection of the ribose conformer populations for the non-methylated and methylated systems clearly reveals that 7-methylation has a decisive influence. The data show that this causes 13-24% increase in ³E conformers and the increase closely follows the number of phosphate groups introduced. It is important to note that 7-methylated di- and triphosphates tend to populate in ³E conformation and this is the first observed case in which purine 5'-nucleotides show a preference for ³E pucker in

Fig. 1 The sequence of mRNA at the 5'-region. The above sequence can be abbreviated as m⁷G^{5'}ppp^{5'}AmpA. Structures in which AmpA has been replaced with any of the common bases have been observed in mRNA. The conformational nomenclature χ , ψ , ϕ , ω , ϕ' , ϕ'' and so on follow definitions according to IUPAC-IUB. In the present paper a letter 'c' is inserted to distinguish between the cap region and the AmpA region. Thus χ_c stands for sugar base torsion of m⁷Gp- and χ_1 and χ_2 for the Amp- and -pA parts respectively. Non-standard abbreviations such as G^{5'}p, G^{5'}pp are used for 5'-GMP, 5'-GDP and so on in accordance with their wide use in the original papers¹⁻¹¹.



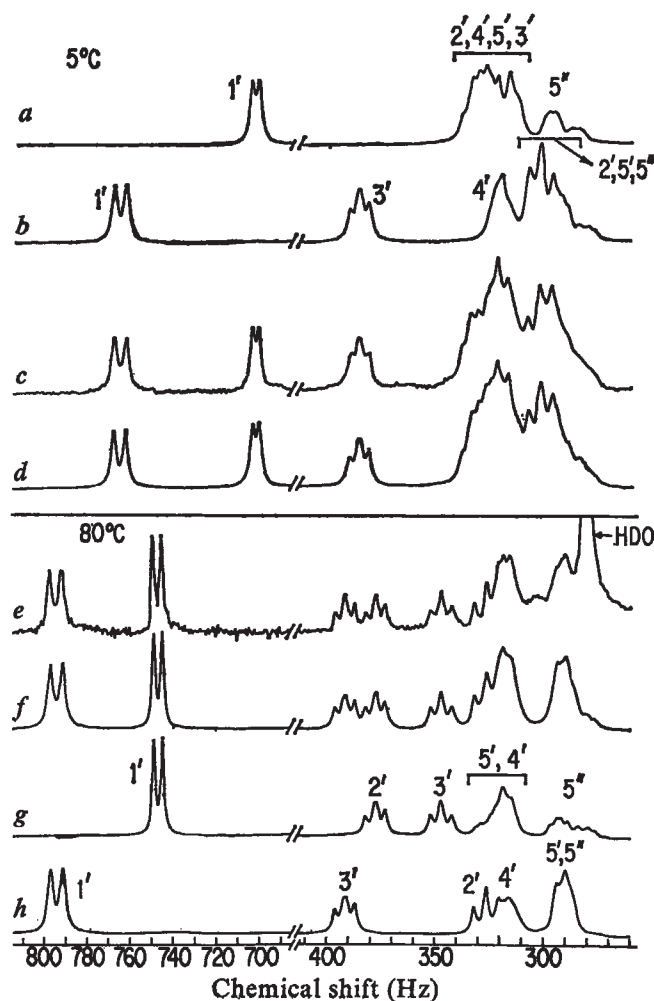


Fig. 2 The observed 270-MHz ^1H NMR spectra of $\text{m}^7\text{G}^{5'}\text{ppp}^{5'}\text{Am}$ at 5°C (c) and 80°C (e). Both spectra were analysed by computer simulation of $\text{m}^7\text{G}^{5'}\text{p}-$ (a and g) and $-\text{p}^{5'}\text{Am}$ parts (b and h) separately (a and b at 5°C and g and h at 80°C) and combining them to produce complete simulation at 5°C (d) and at 80°C (f). The chemical shifts are Hz relative to tetramethylammonium chloride.

aqueous solution. In the 7-methylguanosine series 5'-phosphorylation has no effect on ribose conformation, in the contrast to the 7-methylguanosine series. Changing the ionisation state of the phosphate causes only a slight shift to ^3E pucker for $\text{m}^7\text{G}^{5'}\text{p}$. In the case of di- and triphosphate this has an opposite effect, that is, ^2E population increases. With respect to the common derivatives only the diphosphates are sensitive to ionisation changes.

The data indicate that irrespective of whether mono-, di- or triphosphate, or whether 7-methylated or not, all nucleotides show an overwhelming preference for $\text{g}'\text{g}'$ orientation ($\phi_c = 180^\circ$) about $\text{C}5'-\text{O}5'$. There is, however, a drastic change on conformational preference about $\text{C}4'-\text{C}5'$ in going from 7-methylated nucleoside to corresponding nucleotide. The population of gg orientation ($\psi_c = 60^\circ$) significantly increases on 5'-mono-, di- or triphosphorylation of 7-methylguanosine. A possible reason for this overwhelming increases in gg population is the electrostatic interaction between the positively charged N7 and the negatively charged phosphate groups. In the gg conformation the distance between the phosphate oxygens and N7 is minimum compared with that in the alternate gt ($\psi_c = 180^\circ$) and tg ($\psi_c = 300^\circ$) conformers. The phosphorus chemical shift data summarised in Table 2 clearly substantiate this electrostatic interaction. Particularly noteworthy is the substantial difference in chemical shifts between the γ phosphorus of $\text{m}^7\text{G}^{5'}\text{ppp}$ and $\text{G}^{5'}\text{ppp}$.

It should be noted that $\text{m}^7\text{G}^{5'}\text{p}$, $\text{m}^7\text{G}^{5'}\text{pp}$ and $\text{m}^7\text{G}^{5'}\text{ppp}$ exist

with almost exclusive preference for the gg orientation about $\text{C}4'-\text{C}5'$. This presents a drastic contrast to the situation in $\text{G}^{5'}\text{p}$, $\text{G}^{5'}\text{pp}$ and $\text{G}^{5'}\text{ppp}$, all of which exist as a conformational blend in which the gg population range from 67–77%, the remaining being the alternate gt and tg conformers. This is the first time a nucleotide in aqueous solution has been shown to exist with a rigid geometry about $\text{C}4'-\text{C}5'$. The data thus point out the impact of 7-methylation on the conformational freedom about $\text{C}4'-\text{C}5'$ bond in the guanosine 5'-nucleotides. This is a general phenomenon associated with 5'-purine nucleotides and not something endemic to the guanosine system is illustrated by the data on m^7I and $\text{m}^7\text{I}^{5'}\text{p}$ (Table 1).

X-ray and NMR data and theoretical calculation on mono- and dinucleoside monophosphates (see ref. 18 and refs therein), have shown that the preferred glycosidic torsion in these systems lie in the anti domain. In the 7-methylguanosine series the anti domain is congenial for the electrostatic interaction discussed above.

Conformational properties of 2'-O-methylated monomers

The data in Table 1 for the 2'-O-methylated nucleosides and nucleotides reveal that at the monomer level 2'-O-methylation has only very little influence on the conformational preferences of the ribose ring, $\text{C}4'-\text{C}5'$ and $\text{C}5'-\text{O}5'$ bonds. They, in general, exist as a conformational blend in which ^2E pucker, gg and $\text{g}'\text{g}'$ conformations are preferred. The influence of the ionisation of phosphate on the chemical shifts of base and ribose protons (data not given) reveal that in this system the base occupies anti domain.

Conformational properties of the dimer $\text{m}^7\text{G}^{5'}\text{ppp}^{5'}\text{Am}$

The conformational parameters (Table 1) indicate that $\text{m}^7\text{G}^{5'}\text{p}-$ and $-\text{p}^{5'}\text{Am}$ of the dimer show a significant preference to orient the $\text{C}4'-\text{C}5'$ and $\text{C}5'-\text{O}5'$ backbone in the classically staggered conformation in which $\psi = 60^\circ$, $\phi = 180^\circ$. But, $\text{m}^7\text{G}^{5'}\text{p}-$ component prefers ^3E sugar pucker and $-\text{p}^{5'}\text{Am}$ component prefers ^2E sugar pucker. Comparison of the conformational data for the dimer with that of the monomers at pH 5 (Table 1) clearly indicates that with respect to the local conformations about the sugar ring, $\text{C}4'-\text{C}5'$ and $\text{C}5'-\text{O}5'$ bonds the monomers essentially conserve their conformation in the dimer. There is a small but real shift towards ^3E pucker in the dimer for the $\text{m}^7\text{G}^{5'}\text{p}-$ part, however. This small shift may indicate a reduction in the χ_c of the dimer compared with that of the monomer, because of the conformational nexus between ribose pucker and χ_{CN} (ref. 18).

Information about the molecular topology and intramolecular order of the dimer can be obtained from comparison of the chemical shift data for monomers and the dimer. These dimerisation data are presented in Table 3. The data clearly show that the two nucleotidyl units at the 5' ends of the triphosphate bridge interact with each other, changing the effective field felt at the various protons. As a result of this interaction the chemical shifts of the two residues undergo drastic changes. The protons which show negligible changes are only $\text{H}4'$, $\text{H}5'$ and $\text{H}5''$ of the $\text{m}^7\text{G}^{5'}\text{p}-$ residue. The $\text{H}5'$ and $\text{H}5''$ of the $-\text{p}^{5'}\text{Am}$ residue undergo shifts to

Fig. 3 The two perspectives, drawn using ORTEP, of $\text{m}^7\text{G}^{5'}\text{p}$. The left one shows the 'B' side of purine and the right one the 'A' side. The ribose is ^3E , $\psi = 60^\circ$, $\phi = 180^\circ$ and $\chi_c = 60^\circ$. This is the most preferred conformation of $\text{m}^7\text{G}^{5'}\text{p}$.

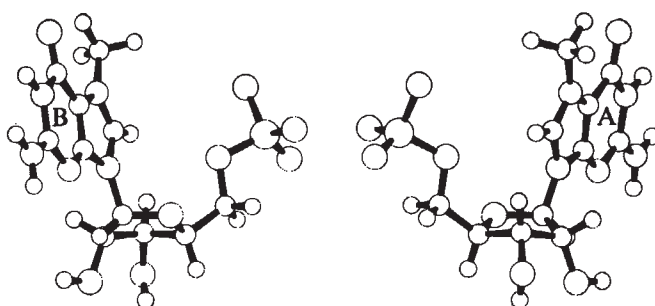


Table 1 Conformational parameters for various components of 5'-terminus of mRNA and corresponding analogues in D₂O

Common nucleosides and nucleotides	pH	% conformational preferences about various bonds					
		C5'-O5' g'g'↔g'/t'	C4'-C5' gg↔g/t	Ribose ³ E↔ ² E			
G	7.2	—	—	69	31	38	62
G ^{5'} p	8	75	25	67	33	36	64
	5	71	29	71	29	37	63
G ^{5'} pp	7	71	29	69	31	44	56
	5	74	26	71	29	37	63
G ^{5'} ppp	7	68	32	77	23	36	64
	5	70	30	79	21	37	63
A	8	—	—	75	25	36	64
p ^{5'} A	7	76	21	75	25	38	62
	5	72	28	77	23	39	61
pp ^{5'} A	8	69	31	79	21	47	53
	5	72	28	79	21	40	60
7-methylated derivatives							
m ⁷ G	7	—	—	76	24	51	49
m ⁷ G ^{3'} p	7	77	23	97	3	54	46
	5	74	26	91	9	60	40
m ⁷ G ^{5'} pp	7	76	24	100	0	65	35
	5	78	22	100	0	53	47
m ⁷ G ^{5'} ppp	7	75	25	100	0	60	40
	5	76	24	100	0	51	49
m ⁷ I	7	—	—	77	23	56	44
m ⁷ I ^{5'} p	7	79	21	95	5	57	43
	5	72	28	98	2	58	42
2'-O-methylated derivatives							
Am	7	—	—	78	22	33	67
p ^{5'} Am	7	73	27	75	25	35	65
	5	70	30	77	23	38	62
pp ^{5'} Am	7	73	27	76	24	40	60
	5	72	28	79	21	36	64
Gm	7	—	—	69	31	37	63
p ^{5'} Gm	7	74	26	73	27	35	65
pp ^{5'} Gm	7	69	31	76	24	42	58
ppp ^{5'} Gm	7	69	31	66	34	35	65
Dimer m ⁷ G ^{5'} ppp ^{5'} Am							
m ⁷ G ^{5'} p-	7 (5 °C)	81	19	100	0	61	39
	7 (80 °C)	75	25	90	10	51	49
-p ^{5'} Am	7 (5 °C)	74	26	88	12	40	60
	7 (80 °C)	72	28	79	21	40	60

Concentration is 0.02–0.07 M for the monomers except for G(0.004 M) and 0.007 M for m⁷G^{5'}ppp^{5'}Am. The temperature in all cases was 19 ± 1 °C unless otherwise stated.

lower field. The remaining protons of both residues are shifted to higher field by as much as 60 Hz. From the observation that H8 and H2 of the -p^{5'}Am moiety and 7-CH₃ group of m⁷G^{5'}p- moiety are shifted to higher field it is immediately apparent that the two aromatic systems interact with each other as probably in a parallel stacking.

It should be emphasised that the interaction between bases takes place while the individual nucleotidyl units maintain very similar conformation for ribose, C4'-C5' and C5'-O5' bonds as in the monomers. Hence this intramolecular stacking interaction must be made feasible by torsional variation around the phosphodiester bonds of the triphosphate bridge. To describe the stacking possibilities between the two ring systems it is necessary to label the sides of purine moieties by the letters A and B as has been done before^{20–22}. These are shown in Fig. 3 for m⁷G^{5'}p. There are four possible types of stacking: (1) A-A stacking. A surface of m⁷G^{5'}p- moiety faces the A surface of -p^{5'}Am moiety (Fig. 4a). (2) A-B stacking. A surface of m⁷G^{5'}p- moiety faces the B surface of -p^{5'}Am moiety (Fig. 4b). (3) B-A stacking. B surface of m⁷G^{5'}p- moiety faces the A surface of -p^{5'}Am moiety (Fig. 4c). (4) B-B stacking. B surface of m⁷G^{5'}p- moiety faces the B surface of -p^{5'}Am moiety (Fig. 4d).

In A-A and B-B stacking the O1' of both ribose rings are oriented in opposite direction whereas in A-B and B-A stacking they are oriented in the same direction (Fig. 4). The four stacking arrangements depicted in Fig. 4 are generated while maintaining the same conformation for the ribose rings, C4'-C5' and C5'-O5'

bonds, and the only changes are in χ_c , χ_1 , ωC_1 , $\omega' C_1$, ωC_2 , $\omega' C_2$, ωC_3 and $\omega' C_3$.

The observation that the base protons of -p^{5'}Am moiety and 7-methyl group of m⁷G^{5'}p- moiety are shielded (Table 3) can be rationalised on the basis of any of the above stacked structures or on the basis of a blend of all the four forms. One should, however, be able to make a distinction by following the shift trends of interior protons such as H1', H2', H3', H4', H5', and H5''. The shifts of H5' and H5'' could be affected by torsional changes in the phosphate backbone as a dimer is formed from the monomer, and hence are of little use in the determination of stacking interactions from ring current considerations. The H1' chemical shift of both residues will be highly sensitive to χ_{CN} variation and the observed upfield shift of H1' (Table 3) is an indication of reduction in χ_{CN} .

Table 2 Chemical Shift data (Hz) of phosphorus in guanosine derivatives

Compounds	α	β	γ
G ^{5'} pp	556	410	—
m ⁷ G ^{5'} pp	566	428	—
G ^{5'} ppp	566	999	400
m ⁷ G ^{5'} ppp	576	1014	423

Shifts are given relative to internal trimethylphosphate, (CH₃O)₃OP. Concentration is 0.05 M, pH 7.0 at 19 ± 1 °C. 40.5 MHz NMR system.

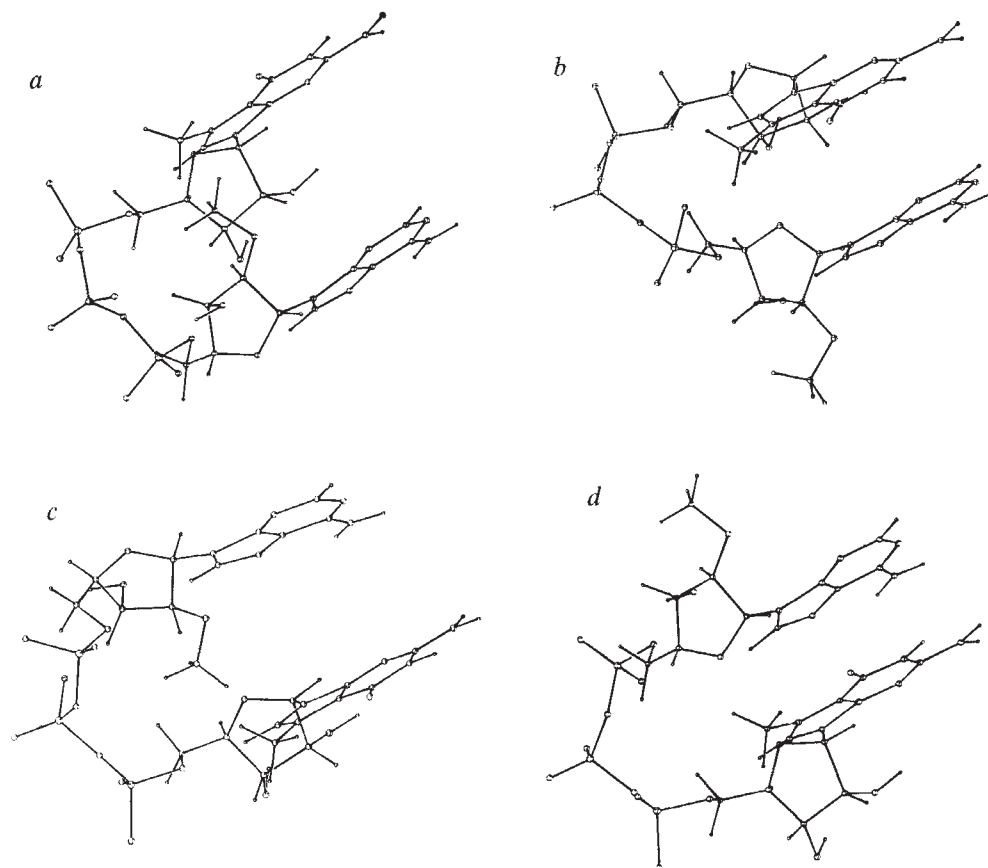


Fig. 4 The four basic stacked arrays A-A (a), A-B (b), B-A (c) and B-B (d) possible for $m^7G^{5'}ppp^{5'}Am$. The perspectives were generated using ORTEP. In all the projections the ribose of the $m^7G^{5'}p$ - part is 3E and that of the $-p^{5'}Am$ part is 2E , further $\psi_c = 60^\circ$, $\phi_c = 180^\circ$, $\phi_1 = 180^\circ$, $\psi_1 = 60^\circ$ and $\phi'' = 180^\circ$. These are the experimentally observed preferred values. The values for χ_c and χ_1 , as well as those for $\omega C1/\omega' C1$, $\omega C2/\omega' C2$, $\omega C3/\omega' C3$ cannot be obtained directly from NMR data because of the lack of appropriate nuclei to produce the desired coupling constants. But the data clearly reveal that the bases are in the antidiagonal and the orientation of the phosphodiester bonds allow stacking interactions. A search in the conformation space produced a set of values for χ_c , χ_1 , $\omega C1/\omega' C1$, $\omega C2/\omega' C2$ and $\omega C3/\omega' C3$ which can generate the four possible stackings.

(refs 23, 24) on dimerisation. This is anticipated because dimerisation and stacking will necessitate the readjustment of χ_{CN} in the dimer compared to the values in the monomers. It should be noted that in both dimer and monomers the base prefers anti domain but the magnitude of χ_{CN} in the dimer is smaller than that in the monomer. NMR methodology cannot precisely determine the magnitude of χ_{CN} but can only provide relative changes²⁴. If the value of χ_{CN} in the monomer is between 50° and 60° , the value in the dimer will be between 30° and 40° . Hence, one cannot use the shift trends of $H1'$ to monitor the stacking interactions. But, the observation that $H2'$ and $H3'$ of $m^7G^{5'}p$ - and $-p^{5'}Am$ have undergone significant shielding to higher fields can be rationalised only on the basis of the presence of significant populations of A-A stacking. Inspection of perspectives (Fig. 4a-d) clearly reveals that only in an A-A stack these four protons lie inside of the stack. Thus $H2'$ (see Table 3) and $H3'$ of $m^7G^{5'}p$ - moiety can be shielded

by the adenine of $-p^{5'}Am$, conversely $H2'$ and $H3'$ of $-p^{5'}Am$ moiety can be shielded by the guanine ring of $m^7G^{5'}p$ -. This mutual shielding is possible only in an A-A stack. The observed difference in the magnitude of shielding between the sets of $H2'$ and $H3'$ protons of two moieties is merely a reflection of the difference in the ring current fields of adenine and guanine moieties²⁶. The ring current shielding abilities of guanine are less than that of adenine^{25,26}. In the present case the guanine ring contains positive charge and this will further dilute the effect of guanine. The data in Table 3 clearly show that the adenine shields the $H2'$ and $H3'$ of $m^7G^{5'}p$ - considerably more effectively than guanine shields the $H2'$ and $H3'$ of $-p^{5'}Am$. This is indeed expected.

Because of the inherent flexibility of the various phosphodiester linkages, in aqueous solution it may be that the dimer $m^7G^{5'}ppp^{5'}Am$ exists as a conformational blend of the various stacked arrays and extended forms. Our major conclusion is that among the

Table 3 Effects of dimerisation and temperature change on chemical shift

	Dimerisation effect* (Hz)		Temperature effect† (Hz)	
	$m^7G^{5'}p$ -	$-p^{5'}Am$	$m^7G^{5'}p$ -	$-p^{5'}Am$
AH8	—‡	27.6	—‡	18.7
AH2	—	22.8	—	36.1
2'-O-Methyl	—	11.8	—	9.2
7-Methyl	25.7	—	22.8	—
$H1'$	62.6	39.8	44.5	29.0
$H2'$ §	60.6	46.3	43.3	24.5
$H3'$	46.3	28.1	29.6	6.0
$H4'$	1.5	6.9	-10.0	-5.7
$H5'$	1.7	-18.0	2.0	-15.5
$H5''$	-0.7	-11.5	-4.5	-1.0

*Value in monomer minus that in dimer.

†Value for dimer at $80^\circ C$ minus that for dimer at $5^\circ C$.

‡GH8 exchanges with D_2O and could not be located.

Note that even though the data indicate significant high field shift for $H2'$, the theoretically projected^{25,26} shielding of $H2'$ originating from stacking interactions in A-A stacks should be larger than what is observed. This is because, this high field shift is internally compensated by the down-field shift²³ of $H2'$ caused by reduction in χ_c associated with 3E sugar pucker.

various conformational possibilities in aqueous solution the A-A stack predominates and the molecule has an inherent proclivity to form A-A stacked arrays. The temperature data on $m^7G^{5'}ppp^{5'}Am$ summarised in Table 3 closely parallel the dimerisation trends and these observations are essentially in agreement with the findings of Ts'o and coworkers²⁷ that elevation of temperature causes de-stacking and at higher temperatures the dimer tends to approximate to monomer conformations.

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letters to nature

Evidence for a 39-d period in Cyg X-1

THE detection of a 39-d period in Cyg X-1 (HDE226868), as seen in the ultraviolet (ultraviolet-filter) polarisation¹, would have at least one interesting interpretation, that of a third-body orbital period (M. Milgrom and J. Shaham, personal communication). We report here the result of long-term monitoring of the optical polarisation of Cyg X-1², which has been in progress since 1974 at Pine Mountain Observatory, Oregon. Up to early August 1977, over 225 nights of ultraviolet-filter data were obtained. Power spectra of the ultraviolet-filter polarisation computed in March 1977 suggested a peak at the approximate period 39 d, common to the separate power spectra of our two instrumental Stokes parameters² Q and U . The amplitude of the feature corresponds to a peak-to-peak polarisation variation of about 0.25%. The Q and U variations proved to be coherent with each other and to be of a rectilinear type, describable as a simple variation along a certain rotated Stokes parameter Q_0 , defined approximately by $Q_0 = p \cos[2(\theta - 117^\circ)]$. The normalised conjugate parameter $U_0 = p \sin[2(\theta - 117^\circ)]$ shows no significant variation at the 39-d period.

Cyg X-1 has an interstellar polarisation of roughly 5%, on which variations of the order of $\pm 0.3\%$ are superimposed. The variability is partly or largely random in character; the isolation of periodicities, therefore, requires a long-term programme. We have also recorded a slow secular change in p , the degree of polarisation, averaging about $0.00047\% \text{ d}^{-1}$ in the ultraviolet; this has amounted to a shift from an average $p = 4.25\%$ in 1974 to 4.55% in 1977.

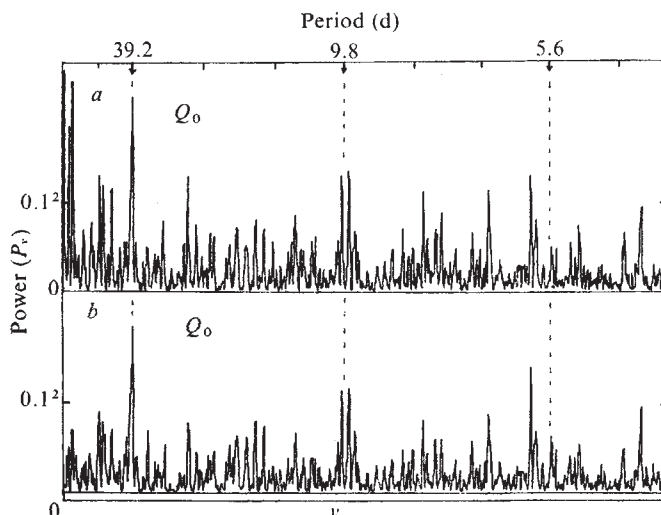
Figures 1 and 2 show power spectra of the ultraviolet-filter polarisation, computed by the bin method², using data from 192 nights spanning the interval September 1974-June 1977. The two spectra in Fig. 1 are of the Q_0 parameter, in which the 39-d effect is most evident. Figure 2 shows the power spectra of Q_0 and U_0 using the slope-corrected data after a mild weighting, in which all data entries (nights) which exhibit excursions ΔQ or ΔU exceeding 0.7% have been assigned relative weights of 0.5 (15 nights are thus de-weighted).

The 39-d peak in Q_0 is the highest peak in all of our power spectra, apart from very long-period structure ($P \geq 400$ d) when the secular slope is not removed as in Fig. 1a. In later results, using 225 nights of data, the contrast of the 39-d feature has improved. A very rapid improvement cannot be expected.

Statistical aspects of the apparent 39.2-d periodicity are summarised in Table 1, which is based on an analysis made with a

packaged statistical program. The latter performs linear regression and yields various data such as F values. We used a multi-function fit to the raw data including: (1) a slope term, to account for the secular change in polarisation; (2) the predominant 39.2-d sinusoid; and sometimes (3) a harmonic, of period 39.2 d/ n , where n is an integer. (The case $n=0.5$ is also included in Table 1.) The likelihood of a null hypothesis for each frequency component, of arbitrary phase but pre-assigned period, is given in column 3. Those probabilities must be multiplied by the number of statistically-independent periods, that is by $N/2$ where in this case

Fig. 1 Power spectra of Cyg X-1 ultraviolet-filter polarisation, based on 192 nights from September 1974 to mid-June 1977. (One data point per night is taken, involving 1-3 h of integration including calibrations.) Computed by simple bin-averaging method of Kemp *et al.*². Such power spectra have slightly poorer signal-to-noise ratios than spectra computed from linear regression, but are less expensive in computer time. Scan uses 1,000 discrete frequencies, six bins. *a*, raw data; *b*, computed from data with secular slope ($\Delta p = +0.0005\% \text{ d}^{-1}$) removed thus suppressing the structure at very long periods (> 200 d). Parameter Q_0 has maximum amplitude at 39.2 d. In all the figures, ordinate is proportional to power, that is to the squared Fourier amplitudes of the polarisation in per cent; abscissa is linear in frequency (inverse period).



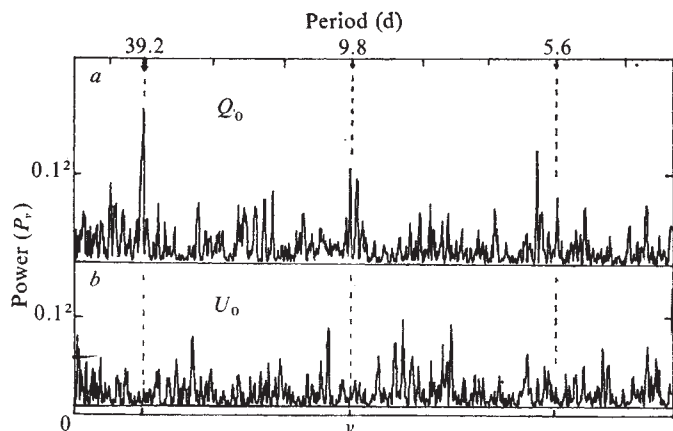


Fig. 2 Power spectra with slope removed, and with data slightly weighted; 19 points with a , ΔQ or b , ΔU (variations from means) given weights 0.5, all others given weights unity. A very slight improvement is noticed in the apparent signal-to-noise ratio. Both Stokes parameters are shown.

$N = 192$. We must also multiply by 2, to account for the two Stokes parameters Q_0 and U_0 . Unbiased probabilities for a random-noise hypothesis are thus given in column 4.

From this analysis, and other criteria, we have concluded that the 39.2-d periodicity is real with a probability which now exceeds 97%.

Of the harmonics, only the fourth (of period 9.8 d) seems to be appreciable. In Table 1 the low amplitudes at 2.8 d and 5.6 d, are of special interest the latter being the well-known binary orbital period.

Figure 3 shows an amplified trace of the Q_0 power spectrum in the vicinity of 39 d. A least-mean-squares fit to the function $(A \sin \Delta \nu / \Delta \nu)^2$ has been made. We find a best-fit period of 39.22 d for the bin-type power spectrum, and 39.30 d for the linear-regression points—or an average of about 39.26 d. To three significant figures³ the binary period of Cyg X-1 is 5.60 d. We thus contemplate the ratio $39.26/5.60 = 7.01$. That is, the 39.2-d period is very likely the exact seventh multiple of the binary period.

Because of the almost integral relationship just shown, one might suspect that the 39-d pattern is an aliasing effect of some kind, but this is unlikely. The test power spectra obtained by applying pure sinusoids to our sampling times have negligible side lobes, less than 1/5 as strong as the central peaks, as we have tested for 39.2-d, 5.6-d and 2.8-d fundamental periods; and furthermore, the Fourier amplitudes at 5.6 and 2.8 d in the observed power spectra are very weak. A confusion with aliases having periods very close to 1.0 d, namely the periods $(1.0 \pm 1/39.2 \text{ d})$, cannot be

Table 1 Cyg X-1, ultraviolet polarisation: components of 39.2-d variation and harmonics from linear regression

Period (d)	Amplitude (in % polarisation)	Simple null probability*	Unbiased null probability†
Q_0 Stokes parameter			
39.2‡	0.129 ± 0.032	1.1×10^{-4}	0.021
39.2§	0.131 ± 0.030	0.7×10^{-4}	0.013
9.8	0.087 ± 0.031	0.0036	(0.69)
5.6	0.041 ± 0.031	0.150	(~1)
2.8	0.045 ± 0.031	0.137	(~1)
78.4	0.035 ± 0.032	0.250	(~1)
U_0 Stokes parameters			
39.2§	0.016 ± 0.034	0.569	(~1)
9.8§	0.017 ± 0.033	0.554	(~1)

*As furnished by statistical program, with pre-assigned period or periods; equals twice the product of probabilities for sine and cosine terms.

†Simple probability multiplied by $(192/2)$, the number of statistically independent frequencies or periods obtainable with 192 data points. Meaningful for small probabilities ($\ll 1$).

‡Based on 3-parameter fit: slope plus 39.2 d sine, cosine.

§From 5-parameter fit: slope plus 39.2 d and 9.8 d sines, cosines.

||From 5-parameter fit: slope plus 39.2 d and harmonic or sub-harmonic sines, cosines.

ruled out, because our observations are always separated by about 1 d or more. We discount that possibility.

As yet the 39-d periodicity has not been detected in other properties. A search of the X-ray flux records does not show such a period (S. S. Holt, and E. N. Walker, personal communications); but the X-ray variability is known to have at best only a tenuous connection with the optical behaviour⁴. Virtually all of the optical photometry of Cyg X-1 has been performed in blue filter (blue light). There is no indication of a 39-d period in the blue photometry (E. N. Walker, and I. G. Nolt personal communications). On the other hand, it is likely that the 39-d effect is

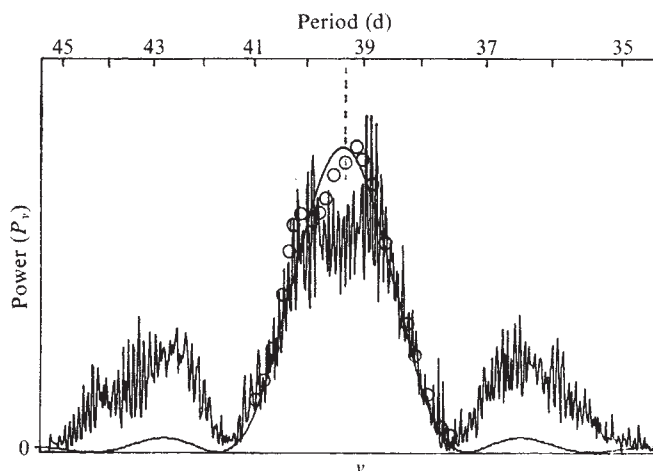


Fig. 3 Local power spectrum, Q_0 parameter, around 39-d period. Jagged curve² is generated by bin-analysis program; circle points were obtained one at a time from multiple regression program including a slope plus sine and cosine (for each period). A curve fit to the diffraction-like function is shown.

wavelength-dependent and is absent in the blue-visible: the ultraviolet-blue-visible polarimetry of 1975² indicate Q_0 amplitudes of $\leq 0.06\%$ in blue and $\leq 0.08\%$ in visible. Ultraviolet photometry, currently under investigation at our observatory, may answer the question of a photometric counterpart of the polarisation period.

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Possible 39-d polarisation period in Cyg X-1

KEMP^{1,2} has reported that the power spectra of the Q and U Stokes polarisation parameters, for the U band radiation from Cyg X-1, show a $\sim 3\sigma$ peak at 39.26 ± 0.10 d. The peak was found to represent a modulation in which Q and U vary, in phase, at 0.27% peak-to-peak total polarisation. While this was reported to be the strongest U band periodicity in the frequency range considered, it was not found in the B and V bands (ref. 3 and J. Kemp, personal communication). Further

observations are necessary to set limits on this modulation for the various radiation bands. This letter suggests that such variable polarisation component may arise from those optical photons which, after being radiated by the disk, are Thomson scattered into our line of sight by the flared material at the outer parts of the disk and in the incoming flow⁴. Details will be published elsewhere⁵. Here we note that, with a favourable ratio of electron scattering opacity to absorption opacity and with a scattering depth $\tau_{es} \sim 1$, such scattering can polarise the photons to a high degree ($\sim 20\%$); we also note, that the in-phase variability of Q and U can come about if the azimuthal structure of the scattering region does not have a 39.2-d variation (for example, if the disk is always axially symmetrical), while the r, z structure does have sufficient variability to change the amount of scattered photons relative to the total flux and hence to produce a variation in total polarisation.

Matter from the primary which flows through L1 to join the outer disk assumes, on a Keplerian time scale, such constant azimuthal structure, which we may call 'the scattering rim'. r, z -Structural variability can then come about by a modulation in L1 overflow. The latter can be induced by tidal interaction with an eccentric, 39-d periodic motion of a third, light, star around the Cyg X-1/HDE 226868 system, in a way similar to that suggested previously for the Her X-1/HZ Her system⁶.

The L1 point for the binary system is located $\sim 10\text{--}16 \times 10^{11}$ cm from the compact object, for the accepted range of binary masses. If the rim is located at a distance of $r_{11} \times 10^{11}$ cm and, for simplicity, extends over 0.2×10^{11} ($\alpha/0.2$) r_{11} cm in the r and $\pm z$ directions, then its transversal optical scattering depth is $\tau_{es} \sim 3(\alpha/0.2)^{-1} \psi r_{11}^{-2} \dot{m}_0$, where $\dot{m}_0$ is the average accretion rate, in units of $10^{-8} M_{\odot} \text{yr}^{-1}$, and ψ is the mass of the 'rim' in units of the total mass accreted during a 39-d period. In steady state, the inward drift time of the 'rim' is of order ~ 39 d and we expect $\psi \lesssim 1$ for a large flow modulation. Due to the non-hydrostatic nature of the rim⁷ and its heating⁸, we expect α to be larger than the angular size calculated for hydrostatic disks. The condition $\tau_{es} \sim 1$ thus seems to be compatible with current understanding of disk and mass flow (when $\tau_{es} > 1$, (but not $\gg 1$) polarisation by backscattering could still occur).

For U band photons, κ_{es} dominates at densities

$$\rho < 2 \times 10^{-10} T_4^{1/2} \text{g/cc} = \rho_c \quad (1)$$

where T_4 is the temperature in 10^4 K. On rewriting equation (1) as $T_4 \geq 0.5 r_{11}^{-2} (\alpha/0.2)^{-2} \tau_{es}^2$, we note that this condition is also likely to be satisfied, since rim cooling drops sharply when $T_4 < 1$ due to the decreasing ionisation, and since the rim is being constantly heated by the incident X-ray flux and the jet-disk shock.

Due to uncertainties in efficiency and total luminosity of Cyg X-1 we can estimate only roughly the U luminosity, $L_{u,d}$, in the direction of the rim. To produce a 0.27% total polarisation with 20% scattering polarisation we must have $L_{u,d} \sim 0.1(\alpha/0.2)^{-1} L_{u,p} \sim 0.2(\alpha/0.2)^{-1} L_{x,d}$, where $L_{u,p}$ is the primary luminosity and $L_{x,d}$ is the 2–6 keV X-ray luminosity⁹. Since most of the optical luminosity is due to X-ray heating¹⁰, we do expect a suppression of B and V rim scattered flux, hence of 39-d B and V polarisation modulation, relative to the U band. This is because such optical surface emission has a strong limb brightening effect¹¹, especially at glazing angles, and may develop a marked inverted Balmer jump (higher intensity for wavelengths below the Balmer edge). Some support for this property of heated surface emission is furnished by indirect observations of the continuum emission from the disk in SMCX-1 (ref. 12).

The existence of a modulated rim structure can be tested by further observations: The hard X-ray (> 10 keV) intensity and polarisation should be modulated at 39 d, with respective peak-to-peak amplitudes of 70aa% and 15aa%, where a is the albedo. The average polarisation for $\alpha \sim 0.2$ and $a \sim 0.5$ is comparable to the predicted^{5,13} and observed¹⁴ constant

polarisation of hard X rays. The corresponding amplitudes for soft X rays should be smaller due to their increased absorption. Furthermore, a 0.015 mag peak-to-peak 39 d modulation in U intensity is expected, with a smaller amplitude for the B and V radiations. All of these modulations should occur in phase, and, to the extent that the rim structure changes during a high-low transition in Cyg X-1¹⁵, their amplitudes should change too.

The existence of a third star can be further tested by observations of additional periodicities in intensities and polarisations. Aside from smaller amplitude binary or half binary periods, periods associated with the variable tidal forces are expected. The latter form a large set of sums of integral multiples of $\frac{1}{2}(\omega_b - \omega_3)$ and the 39 d frequency, where ω_b is the binary frequency and the ω_3 's are instantaneous frequencies of the third stellar motion. Such tidal modulations will only show up as flow modulations if the time responses at L1 and during the free-fall, circularisation and inward rim diffusion are sufficiently small. The proximity of the observed 39.2-d period to an integral multiple of half a binary period (14×2.8 d) may be due to the eccentricity of the binary orbit or may be related to the forbidden binary phase for X-ray turn-ons in Her X-1 (see ref. 6). We also expect longer periods, associated with modulations in binary eccentricity and precession of its nodes.

Similar polarisation measurements for the Her X-1/HZ Her system may distinguish between models suggested for its 35-d period. For example, if U and Q are found there too to vary in phase, this may rule out a precessing disk model.

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The 39-d period in Cyg X-1

KEMP *et al.*^{1,2} recently claimed to have discovered a 39-d period in the linear polarisation of the U band light for HDE 226868, the optical counterpart of Cygnus X-1. Within the errors their period is exactly seven times the 5.60-d orbital period of this binary system. Based on this result Milgrom and Shaham³ produced a model for Cyg X-1 involving a three-body system in which the third component is in a 39-d orbit about the 5.6-d X-ray binary system. This note draws attention to the absence of a 39-d period in other data on this star.

Walker and Rolland Quintanilla⁴ have recently completed an analysis of 349 nights of B band data obtained in 1972–76.

Two methods were used to search these data for possible periods. The first was based on the method of Lafler and Kinman⁵ and the second on the Fourier technique developed by Grey and Desikachary⁶. Neither method shows any evidence for a period of 39 d or at one half or double that period. A realistic upper limit can be set to any modulation of the blue light of this star at any of these periods of 0.001 mag., that is, 0.1%. This has to be compared with the modulation at 2.8 d (half the orbital period) of over 2%.

A literature search for periods in 215 radial velocities for this star⁷⁻¹⁵ allows us to put an upper limit on any 39-d period of $<10 \text{ km s}^{-1}$ compared with over 60 km s^{-1} for the variations with the 5.60-d orbital period.

We have also searched the X-ray flux measurements for periods using the Grey and Desikachary⁶ method. The data are from the All Sky Monitor (2–6 keV) and the Sky Survey Instrument (2–18 keV) on the Ariel V satellite. Combined data from both experiments give almost complete coverage at one satellite orbit interval ($\sim 100 \text{ min}$) over the 590 d before March 1977 including both high and low states of X-ray activity. The high state data are characterised by massive (approximately fourfold) modulation of an apparently random character and an upper limit of $\sim 10\%$ is the best that can be placed on any 39-d modulation. But, the much more extensive low state data, where the overall modulation is much smaller, give an upper limit of $\sim 3\%$ to any 39-d modulation. In both cases the power at $\sim 39 \text{ d}$ is significantly less than that at nearby frequencies and in neither case does the power spectrum suggest any significant 39-d period.

We believe that the reality of this period and the subsequent model must be in doubt. This is because of the marginal nature of the evidence for a 39-d period in the U-band polarimetry and the upper limits to modulation with this period, or its nearest harmonics, in the B band, radial velocity and low state X-ray data.

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Mercury in surface waters of seas around the United Kingdom

A SURVEY of the concentration of mercury in the surface waters of the seas around the UK, has been made as part of the Ministry of Agriculture, Fisheries and Food's overall programme of investigation into the distribution of heavy metals in the marine environment. Using a cold vapour–

gold trap method on board a research vessel, thus obviating the necessity to store the samples with consequent problems of loss and/or contamination, both 'reactive' and 'total' mercury concentrations have been measured. The results reported here show clearly the major sources of emission. The possible reasons for differences between these results and those of some other investigators are also discussed.

Preston *et al.*¹ have described a pilot survey carried out to measure the concentrations of selected heavy metals in seawater, suspended matter and biological indicators from British coastal waters. Since then analytical methods have been refined and more surveys carried out. Recently, because of the significance of possible mercury pollution and its effect on man, surveys have been carried out to measure the mercury levels in UK coastal waters. Because of the problems of loss of mercury and contamination of samples with mercury^{2,3}, we have analysed such samples immediately after collection. The technique described here is designed for use on board a research vessel.

The sample, collected either by plastic bucket or Niskin bottle and not filtered, is immediately transferred to a 2.5 l glass bottle, previously checked for contamination, and acidified by adding 10 ml of concentrated sulphuric acid per l of sample. A 100 ml aliquot of this is taken for analysis. The mercury is swept from solution, after reduction with stannous chloride, by bubbling nitrogen through the sample and collecting the mercury on gold wire chips. The mercury is then determined by heating the gold to high temperature, and sweeping the mercury through a Coleman MAS 50 mercury analyser. The resultant absorption peak is recorded on a strip chart recorder set at about a $\times 30$ expansion of the MAS 50 output. The peak height is measured and compared with a standard calibration line produced either by internal standardisation, or addition of mercury standards to a previously reduced sample. The practical detection limit is about 0.5 ng l^{-1} and the coefficient of variation at 1 ng l^{-1} level is about $\pm 10\%$. The analysis takes 10 min from the time the sample arrives on deck.

The results of the analysis of samples taken on two cruises during 1976 are given in Fig. 1. The cruises were carried out between 2 and 16 January, a period of extremely stormy weather, and between 25 May and 14 June, a period of prolonged calm weather, when the suspended load was abnormally low. The areas have been chosen arbitrarily, and the individual high spots have not been included in the calculation of the mean value for each area. Figure 2 shows contours derived from the data summarised in Fig. 1 and the positions of the sampling stations. The main areas of mercury contamination are apparently in the Thames Estuary, Liverpool Bay, the area off the Humber Estuary and, to a lesser extent, the Bristol Channel and the mouths of the Rhine and Elbe rivers. The data from areas A, B, C, D, H and L do not show any clear cut contouring.

The exact forms in which mercury exists in seawater are in doubt, but it is likely that as well as being present as an anionic chloride complex, it will also be complexed by organic material both in solution and in suspended matter. How filtering affects the mercury content of the sample is uncertain, so an environmentally artificial, analytical delineation between 'reactive' and 'total' mercury has been conveniently adopted. The 'reactive' mercury is defined as that which is available for reduction by stannous chloride from samples acidified to about pH 1. The 'total' mercury is that made available to reduction by stannous chloride, following oxidation of the sample with ammonium persulphate. Chlorine, produced during the oxidation, interferes with the measurement, and is removed by a ferrous sulphate scrubber after the reduction step. The mean values obtained for total mercury levels are given in Table 1. The values for the areas H, I and J are not available due to difficulties experienced with the oxidation stage during the early part of each

cruise. In every case an increase in the mercury measured after oxidation was observed.

Samples collected in the open ocean areas, acidified and analysed immediately, stored for 1 month in glass and then reanalysed, gave similar results for both the 'reactive' and the 'total' mercury. One sample, however, collected during 1975, in an area of high suspended load and high mercury contamination gave results of 8 ng l^{-1} and 73 ng l^{-1} for 'reactive' and 'total' mercury respectively, and 1 month later 58 ng l^{-1} and 75 ng l^{-1} respectively. Samples which have been treated by boiling with acid alone have also shown an increase in measured mercury, to a level which is not as high as that obtained by oxidation.

In samples collected at offshore stations no attempt was made to distinguish between mercury present in solution and that associated with any particulate material present. But, one sample (collected during 1975, in an estuary where pollution does occur and containing a very high suspended load) was filtered through a $0.22\text{-}\mu\text{m}$ filter membrane; and on analysis 17 ng l^{-1} were found in the filtrate and 950 ng were found in the particulate associated with that 1-l sample.

Conclusions from these data are that the mercury present in seawater samples is complexed to varying degrees and that a measurement of the total mercury present requires a vigorous oxidation step to make the mercury available for reduction by stannous chloride in cold vapour measurement techniques.

Samples collected in open ocean areas acidified and stored in glass containers are stable for at least 1 month. In samples collected in areas of high suspended load, however, a change in the ratio of 'reactive' to 'total' mercury may occur on storage in acid conditions, although the total mercury is unchanged. In addition a large proportion of mercury present in samples of high suspended load is associated with the particulate matter. The fact that apparently higher levels were found in the Irish Sea than the North Sea may be an artefact of the sampling conditions and proximity to the point of discharge. Those in the western and southern

Fig. 1 Concentration of reactive mercury (ng l^{-1}) from two surveys in 1976. Values given are mean concentration (range of values) and no. of samples analysed. Positions marked, for example, $\times 15$ are position of sampling and concentration measured. Where total mercury is also available positions are marked, for example, $\times 15$ (30).

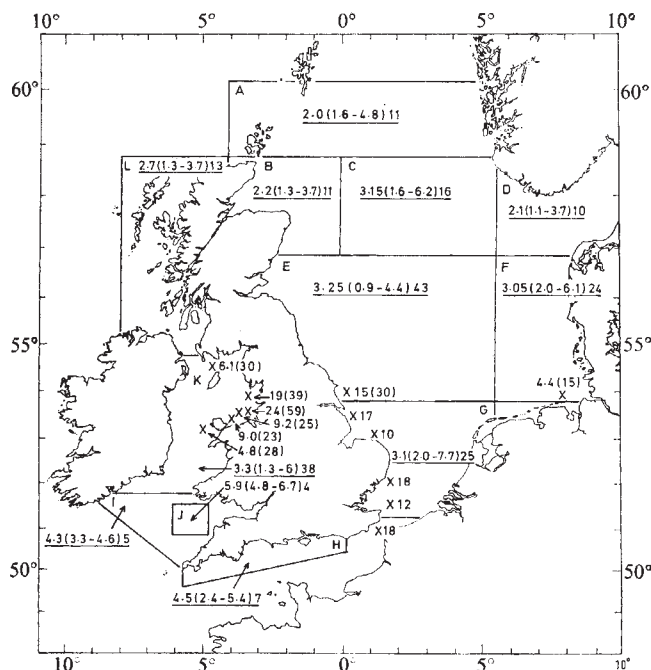


Table 1 Concentrations of total mercury levels where available and ratio of reactive to total mercury for individual areas

Area	Mean total Hg (ng l ⁻¹) (range)	Reactive Total
A	6.1 (3.4–9.5)	0.33
B	6.4 (3.9–11)	0.34
C	7.3 (4.3–12)	0.43
D	6.8 (4.8–9.1)	0.31
E	6.6 (3.5–11)	0.50
F	7.5 (5.2–11)	0.41
G	7.9 (5.6–11)	0.39
K	13 (6.0–22)	0.25
L	9.6 (4.8–20)	0.28

used in the oxidation step, will over a very long period, continue to release mercury into the samples, therefore we use quartz vessels during the oxidation step.

Matsunaga *et al.*¹⁰ measured the 'reactive' mercury concentrations in seawater and reported values of 5 (3.6–5.6) ng l⁻¹ which are in good agreement with the values obtained in this survey, 3.3 (0.9–7.7) ng l⁻¹. They did not, however, oxidise their samples and had they done so higher values would have resulted.

The ratios of 'reactive' to 'total' mercury present are similar to those obtained by Fitzgerald and Lyons⁴ although the concentrations in this survey were of an order of magnitude lower.

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Mafic and ultramafic rocks associated with granites in the Vardar zone

SEVERAL ophiolitic alignments have been classically recognised in the Dinarides and the Hellenides (Fig. 1). The Eastern alignment, located near the Serbo-Macedonian massif in the Vardar zone, shows curious associations of mafic and ultramafic rocks with granites. Here a brief description of the Guevgueli magmatic complex which belongs to the Eastern alignment helps illustrate this peculiarity: petrographic and major element data seem to prove that these associations occur on the border of opening inter-arc basins.

The Guevgueli complex, in Greek Macedonia, is a large thrust slice within Jurassic sedimentary and volcanic formations¹. A fault separates this complex into two petrographically distinct units (Fig. 2)². (1) The Western unit is built up of plutonic rocks—gabbroic cumulates, high Fe and Ti diorites (FeO* 18%; TiO₂, 5%) (FeO*: total iron as FeO), quartz-diorites and rare trondhjemites—separated from lavas by a continuous layer of hypabyssal formations. All these rocks have a very low K₂O content and present a tholeiitic differentiation trend³ with a marked enrichment in FeO* and TiO₂.

(2) The Eastern unit is more varied: it is made up of lavas, hypabyssal rocks forming a sheeted

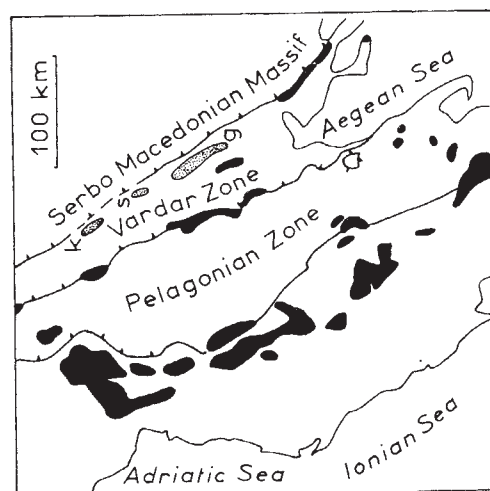


Fig. 1 Structural map of the central part of the Dinarides and the Hellenides. Black areas, ophiolites; stippled area associations of mafic and ultramafic rocks with granites (k, Karadagh; s, Stip; g, Guevgueli).

complex, and gabbroic cumulates cut by abundant magmatic breccias in which dolerites, gabbros, diorites, tonalites, trondhjemites and granites are closely associated. The sheeted complex has a calc-alkaline affinity³, whereas the magmatic breccias present a tholeiitic differentiation trend with enrichment in FeO* and TiO₂. These rocks are in tectonic contact with biotite-orthoclase-sillimanite-cordierite migmatites. A granite massif (the Fanos Granite) is intrusive into the migmatites, the gabbroic cumulates and the magmatic breccias; the biotites of this granite, dated by K–Ar and Rb–Sr methods, are 150 Myr old (Upper Jurassic)⁴.

The Eastern unit seems to result from the juxtaposition of two groups of magmatic rocks. The first one is made up

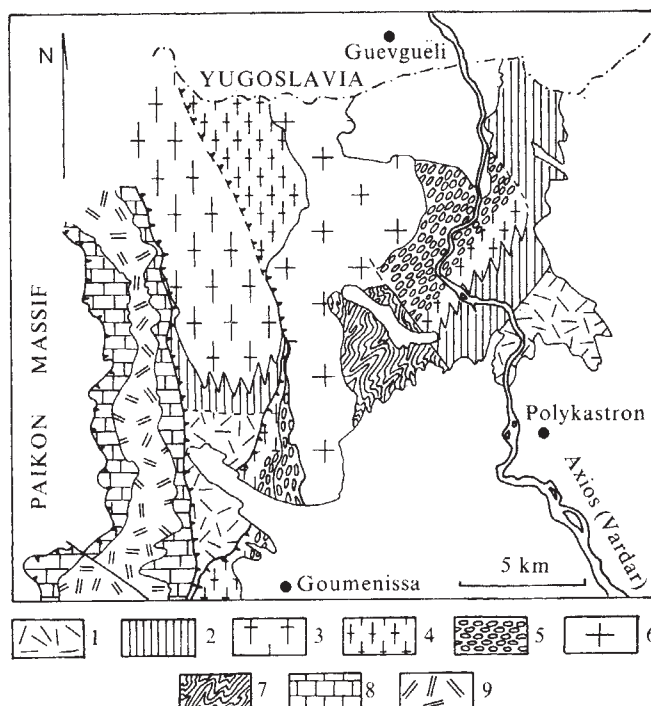


Fig. 2 Geological map of the Guevgueli complex. 1, lavas; 2, hypabyssal rocks; 3, gabbroic formations (Western unit); 4, gabbroic formations (Eastern unit); 5, magmatic breccias; 6, Fanos Granite; 7, migmatites; 8, Mesozoic limestones; 9, volcano-sedimentary formations rich in rhyolites.

of basic, intermediate and acid formations with low K_2O/Na_2O ratios; the second one consists of granitic rocks with $K_2O/Na_2O > 1$. This juxtaposition can be seen in the magmatic breccias, in the sheeted complex which includes microgranitic dykes, and in lavas in which rhyolites are present. These observations lead to the conclusion that the low and high K_2O formations are contemporaneous.

Ultramafic rocks are scarce in the Greek part of the Guevgueli complex, but they outcrop on the other side of the border in Yugoslavia³. Moreover, the Yugoslavian magmatic complexes of Karadagh, near Skopje, and Stip^{6,7},

the TiO_2 content and the FeO^*/MgO ratio, for low values of FeO^*/MgO , in abyssal tholeiites; this positive correlation does not appear, or is very weak, in low K_2O island-arc tholeiites (Fig. 3). The variations of the TiO_2 contents with the FeO^*/MgO ratio in hypabyssal rocks of the Western unit and magmatic breccias of the Eastern unit of the Guevgueli complex, in dolerites and lavas of the Yugoslavian Karadagh complex, and in abyssal tholeiites are comparable (Fig. 3).

The magmatic complexes of the Eastern ophiolitic alignment in the Dinarides and the Hellenides, therefore, show a

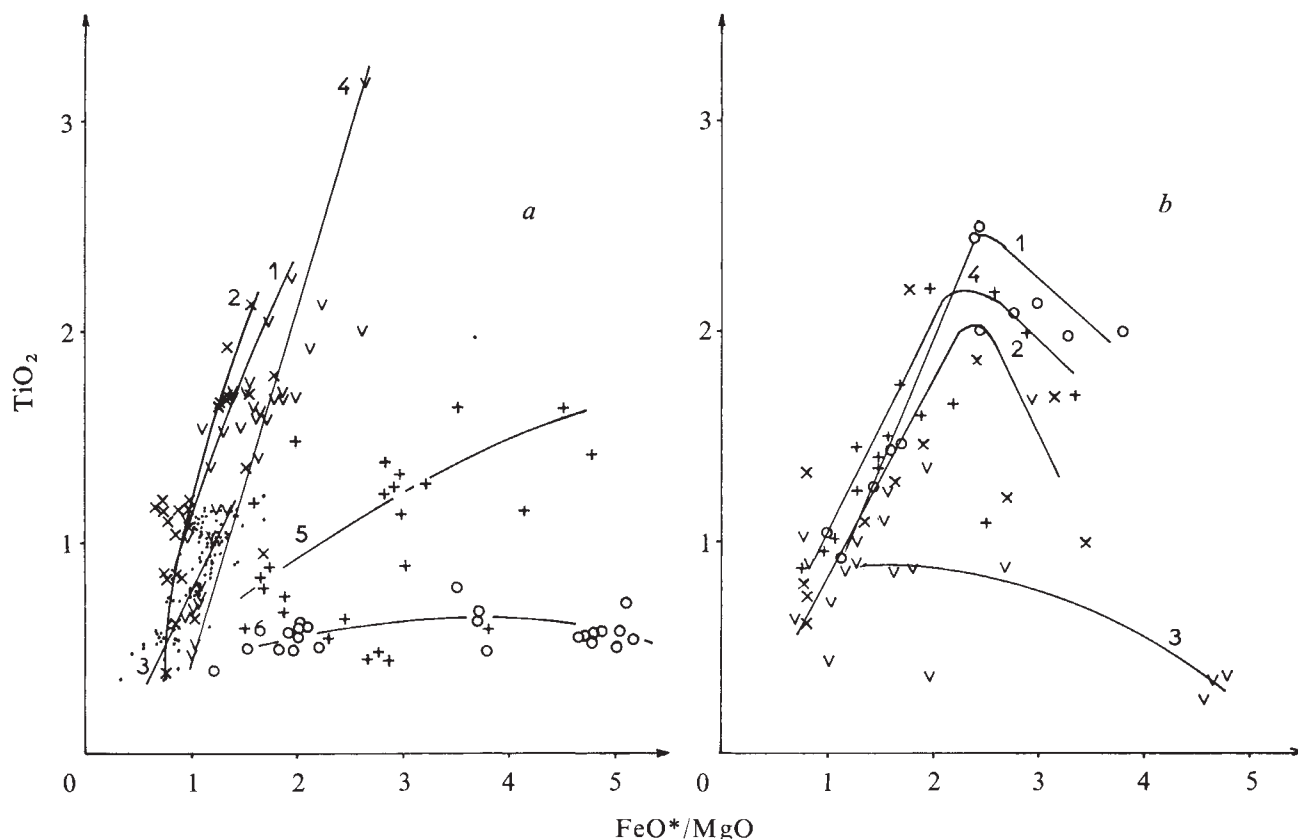


Fig. 3 TiO_2 , FeO^*/MgO diagrams *a*, of recent volcanic rocks; 1, abyssal tholeiites⁸; 2 ($\times$), basalts from the Lau Basin, an interarc basin between the Tonga and the Lau Ridges¹¹; 3 ($\bullet$), basalts from Leg 37 of the Deep Sea Drilling Project (abyssal tholeiites with very low TiO_2 content)¹²; 4 (V), basalts from Iceland¹³; 5 (+), low K_2O tholeiites from Hachijo-Jima, Izu-Bonin Arc¹⁴; 6 ($\circ$): low K_2O tholeiites from Tonga¹⁵. *b*, Of magmatic formations of the Eastern ophiolitic alignment in the Dinarides and the Hellenides: 1 ($\circ$), hypabyssal rocks, Western unit, Guevgueli complex; 2 ($\times$), magmatic breccias, Eastern unit, Guevgueli complex; 3 (V), hypabyssal rocks, Eastern unit, Guevgueli complex; 4 (+), dolerites and lavas, Yugoslavian Karadagh Massif⁶.

located near the Serbo-Macedonian massif, also show close associations of mafic and granitic rocks and include important masses of ultramafic formations (dunites with chromite, lherzolites, harzburgites, pyroxenites).

Thus, all these magmatic complexes exhibit the juxtaposition of distinct associations. The abundance of granites does not seem compatible with an oceanic setting for these rocks. The presence of formations with calc-alkaline affinity (sheeted complex of the Eastern unit) is classically considered to be characteristic of island arcs and active continental margins⁸. Two kinds of magmatic rocks can be compared to the tholeiitic series with low K_2O content of the Guevgueli complex: low K_2O island-arc tholeiites and abyssal tholeiites. The similarity between these formations has caused many discussions on the origin of some ophiolitic complexes. It seems that the TiO_2 content can resolve this problem. The low K_2O tholeiites of island-arcs usually have slightly lower TiO_2 contents than common abyssal tholeiites, but there are many important exceptions⁹. On the other hand, a marked positive correlation can be observed between

close relation in space and time between abyssal tholeiites, island-arc magmatism and granites. Such associations are found on the border of inter-arc basins; so, it seems that the magmatic rocks of the Guevgueli and Karadagh complexes were formed during the creation of inter-arc basins in the Vardar zone. The ultramafic and mafic formations with tholeiitic affinity are relics of the crust and the mantle of inter-arc basins; the calc-alkaline and granitic rocks are the results of the magmatic activity on the borders of these opening basins. This interpretation, if true, is important for our understanding of the structure¹⁰ of the Dinarides and the Hellenides.

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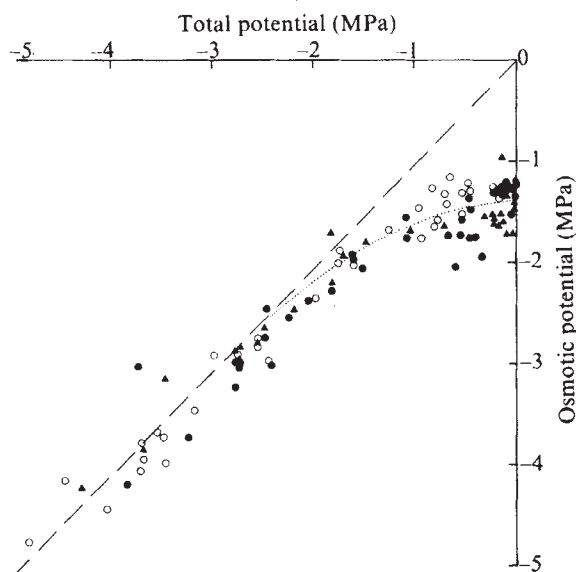
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Differences in osmoregulation between wheat genotypes

SEVERAL recent experiments provide evidence to support the existence of osmoregulation in plants as a means of counteracting water stresses induced by changes in the soil or evaporative environment¹⁻³. Viewed in terms of energy potentials, decreases in the water potential, Ψ , induced by changes in the environment, are immediately offset by decreases in the osmotic potential, π , through an increase in solute content. The turgor potential, P , is thereby maintained since $\Psi = \pi + P + \tau$ (assuming that the matric potential, τ , is small or constant). The importance of this response is that expansion growth, which is affected directly by the turgor potential, is maintained over a range of values of Ψ ². Despite this obvious significance, little is known of the extent of this phenomenon in crop plants in general and in particular of differences between genotypes in the same or closely related species. Here we present evidence indicating substantial differences between several genotypes of wheat; in some osmoregulation was marked, whereas in others it was virtually non-existent.

To examine a wide spectrum of the possible variation, the following genotypes were selected: *Triticum aestivum* spp. *vulgare* (AUS 2067), *T. aestivum* spp. *spelta* (AUS 3843), *T. aestivum* spp. *vulgare* (AUS 3850), *T. durum* (AUS 2816), *T. dicoccum* (AUS 3582) and *T. boeoticum* spp. *aegelopoides* (AUS 90352). After vernalising the plants were grown in pots in a glasshouse with temperatures 20-25 °C during the day and 10-15 °C during the night. Water stresses were produced by withholding water at two stages of development: about 14 d before and 14 d after anthesis. Plants were placed in a controlled environment chamber adjusted to give a flux density of total short wave radiation of 140 W m⁻² at the average plane of the flag leaves and temperatures of 23 °C during the light period

Fig. 1 Relationship of leaf water potential to osmotic potential. The broken line is the line of equality. $\blacktriangle$, *T. durum* (AUS 2816); $\circ$, *T. aestivum* spp. *vulgare* (AUS 2067); $\bullet$, *T. boeoticum* (AUS 90352).



(duration 14 h) and 15 °C during the dark period. Total potential Ψ was measured on samples of the flag leaves using thermocouple psychrometers and π was measured in the same way after the samples had been frozen in liquid nitrogen⁴. Relative water contents were measured using the technique of Slatyer and Barrs⁵.

When Ψ falls in response to a reduction in soil water supply, it is usual for π to fall at a slower rate, such that there is a linear decline in the turgor potential until $\Psi = \pi$. This pattern has been observed for a range of different plant species, including wheat⁶⁻⁸. The response of the genotypes *T. aestivum* spp. *vulgare* (AUS 2067), *T. durum* (AUS 2816), *T. boeoticum* spp. *aegelopoides* (AUS 90352) fit this pattern (Fig. 1). The osmotic potential fell from a value of about -1.5 MPa at full turgor to a value of about -2.5 MPa at zero turgor. This was very similar to the pattern observed in a previous experiment⁷. When osmoregulation occurs we expect π to decrease with Ψ so as to maintain P . This was in fact observed for the genotypes *T. aestivum* spp. *vulgare* (AUS 3850), *T. dicoccum* (AUS 3582), and *T. aestivum* spp. *spelta* (AUS 3843) (Fig. 2). Here, over the range of Ψ for which the leaf had positive turgor, π declined, from an initial value of about -1.5 MPa, at approximately the same rate as Ψ , thus maintaining P . At the end of this period of adjustment, when Ψ had reached about -1.5 MPa and π about -3.0 to -3.5 MPa, π equilibrated rapidly with Ψ . This phenomenon was observed at both stages of development.

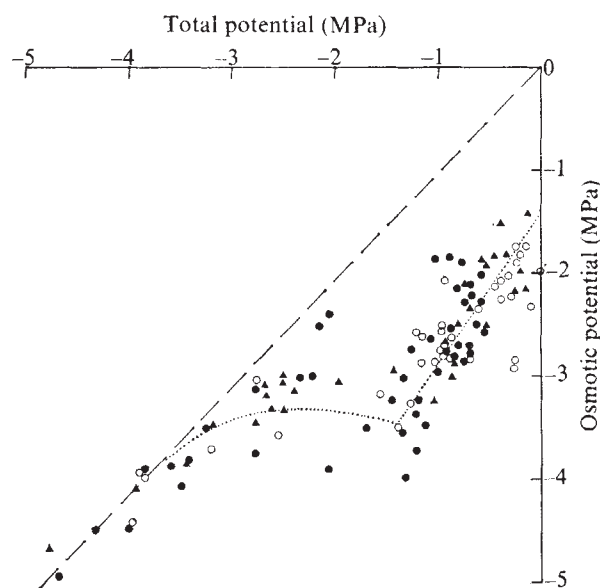


Fig. 2 Relationship of leaf water potential to osmotic potential. The broken line is the line of equality. $\blacktriangle$, *T. aestivum* spp. *vulgare* (AUS 3850); $\circ$, *T. dicoccum* (AUS 3582); $\bullet$, *T. aestivum* spp. *spelta* (AUS 3843).

If the solute content of the leaves did not vary with the relative water content, ζ , then π should be altered by gains or losses of water according to the equation⁹

$$\pi = (\pi_t \zeta_t) / \zeta \quad (1)$$

or if bound water is present, $(\pi + \tau) = (\pi_t + \tau_t) \{ (1-B)/(\zeta-B) \}$ where B is the amount of ζ that is bound, and the subscript t indicates the value at full turgor. If the response is described by equation (1), then the relationship between $\log \pi$ and $\log \zeta$ will be linear. This was in fact so for the genotypes showing no evidence of osmoregulation, an example being *T. durum* (Fig. 3a). A value of π_t of -1.3 mPa was found by fitting a regression line to the data and then using this value in equation (1). The line predicted by this equation was reasonably close to the fitted line (Fig. 3a).

For genotypes showing evidence of osmoregulation, the

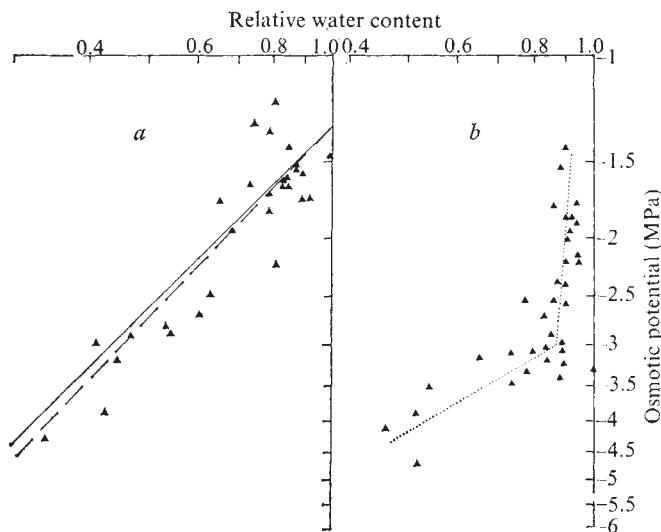


Fig. 3 Changes in the relative water content with osmotic potential for a, *T. durum* (AUS 2816) and b, *T. aestivum* (AUS 3850). The broken line is the line of best fit, the intact line that predicted by equation (1) and the dotted line an interpretation of Fig. 3b.

relationship seemed to consist of two distinct linear phases, as shown for *T. aestivum* (AUS 3850) in Fig. 3b. In the first, which covered the range of π from -1.5 to -3.0 MPa, there was virtually no change in ζ . Since changes in π balanced changes in Ψ over this range (Fig. 2), the relative water content was conserved for changes in Ψ as well. At the end of this phase, there was a linear decline in π similar to that observed for plants of the type shown in Fig. 3a.

This pattern, being bi-modal, cannot be described by equation (1). Also, it is difficult to explain in terms of so called bound water, since the level would have to be about 0.9 in order to explain the first phase, with a rapid change to almost zero in order to explain the second. This would presumably require a sudden decrease in the amount of colloidal material in the leaf tissue. A more plausible explanation may be found if we assume that the solute content changes with decreases in Ψ up to the end of the first phase, that is, that osmoregulation occurs. At the end of this phase it seems that this process ceases and that π then declines with change in Ψ due to loss of water from the tissue and subsequent increase in the solute concentration, that is, according to equation (1). It is not clear from these results what limits solute accumulation, or why it should be present in some genotypes and not others. Indeed, there seems little point in speculating on these issues until more information has been obtained. It is, however, clear that the two types of response can occur in wheat, and that when osmoregulation occurs the turgor potential can be maintained over a substantial range of Ψ —up to -1.5 MPa. More importantly, these results suggest that large genotypic variations in osmoregulation may exist, and that it may therefore be possible to improve the drought hardness of commercial wheat varieties by breeding for this characteristic.

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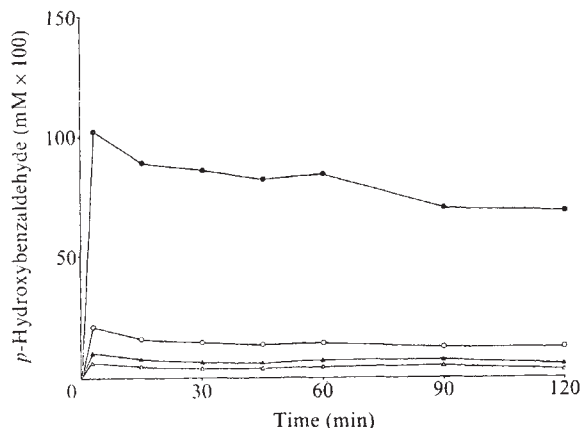
Changes in release rates of cyanide in relation to palatability of *Sorghum* to insects

SORGHUM leaves contain the cyanoglycoside dhurrin located in the cell vacuole¹, and when the tissue is crushed, hydrolysis of dhurrin by an enzyme system probably present in the cytoplasm¹ results in the release of HCN. Cyanogenesis is probably a mechanism to protect plants from being eaten by herbivores^{2,3}. It is generally assumed that this protection is conferred by the toxic effects of cyanide but the mechanism has not been fully investigated and the cyanoglycosides themselves or the other end products of hydrolysis may deter feeding. The degree of liberation of HCN in leaves is dependent on the age and variety of plant, as well as on certain environmental factors^{4–7}. Leaves of young *Sorghum* are often rejected at the first bite by the graminivorous locust, *Locusta migratoria*⁸ but older *Sorghum* is eaten in large quantity. We show here that change in palatability is related to the rate at which HCN is released from the leaf at the time of biting.

Crystals of dhurrin (*p*-hydroxymandelonitrile- β -D-glucoside) (melting point 164° – 168° °C), which liberated HCN when emulsin was added, were extracted⁹ from 8-d-old *Sorghum bicolor* (variety 65D, from Botswana) grown in controlled conditions (18 h light, 25° °C day, 20° °C night). When dhurrin was tested for palatability by *Locusta* nymphs, by absorbing it on to sucrose-impregnated glass fibre papers which *Locusta* normally eat, it had no effect on feeding. The hydrolysis of dhurrin is stoichiometric¹⁰ yielding, in addition to HCN, *p*-hydroxybenzaldehyde: this also had no effect on feeding. During the hydrolysis of dhurrin in freeze-dried *Sorghum* leaves, *p*-hydroxybenzaldehyde at first built up and then began to disappear from the test solution (compare ref. 10): the absorption maximum (in alkaline solution) shifted from 330 nm (*p*-hydroxybenzaldehyde) to 285 nm as the reaction proceeded probably because of accumulation of chelidonic acid¹¹. Attempts to determine any antifeedant activity have proved inconclusive.

The release of HCN from crushed leaves was measured quantitatively by monitoring production of *p*-hydroxybenzaldehyde (Fig. 1). The amount released was very high in young plants and declined with age. On very young *Sorghum* insects

Fig. 1 Formation of *p*-hydroxybenzaldehyde from dhurrin present in *Sorghum* at different stages of development. Fresh tissue (0.5 g) of *Sorghum* 65D aged 8 d, 11.5 cm (●), 15 d, 16 cm (○), 30 d, 24 cm (▲) and 53 d, 40 cm (△) was crushed in distilled water (50 ml) and the mixture was aerated. Samples (5 ml) were withdrawn after 5 min and at 15-min intervals for 2 h, the reaction was stopped by the addition of acid and *p*-hydroxybenzaldehyde was extracted into ether, then into sodium bicarbonate and re-extracted into ether after acidification. The extracts were concentrated to dryness, the residue was redissolved in methanol and the absorbance at 285 nm due to *p*-hydroxybenzaldehyde was recorded.



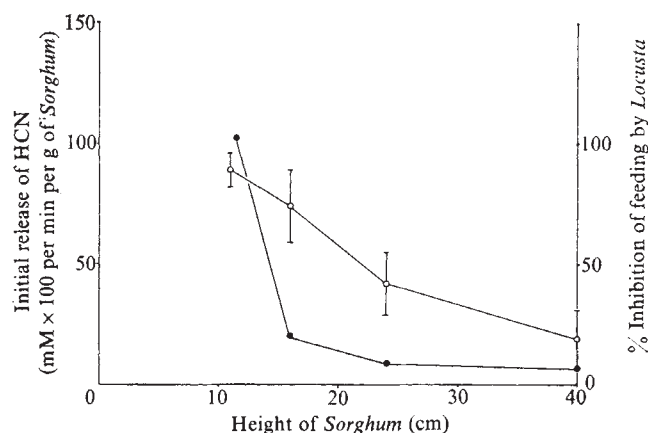


Fig. 2 Release of HCN from *Sorghum* at different stages of development in relation to feeding by *Locusta migratoria migratorioides*. Initial rates of release of HCN from *Sorghum* (●), as would occur on biting, could be calculated from data in Fig. 1 because the hydrolysis of dhurrin yields 1 mol of HCN and 1 mol of *p*-hydroxybenzaldehyde. Percentage inhibition of feeding (○) was found by comparing meal sizes of 5th instar nymphs on *Sorghum* of different ages, with meal sizes on mature, palatable leaves of *Poa annua* used as a control. (Standard deviations are indicated by vertical lines.)

reject the plant quickly; if HCN is the important deterrent it will be the amount released immediately on biting that is relevant rather than the capacity of the plant to produce HCN over a long period. The initial rate of HCN release was calculated for each plant age, and its relationship to the amount eaten in one meal is shown in Fig. 2.

The possibility of a rapid response to HCN was confirmed by introducing hydrocyanic acid into the mouth region of *Locusta*, by means of a previously fitted cannula, when the insect was feeding on a palatable grass. Concentrations as low as 0.01 mM were sufficient to inhibit feeding (Table 1). The amount of HCN initially released from young *Sorghum* (8-d-old) was calculated to be equivalent in concentration to a solution of between 0.5 mM and 1.0 mM hydrocyanic acid and thus would be effective as a deterrent.

Cyanoglycosides have been shown to have no effect on feeding by insects, nor to stimulate feeding at concentrations which occur naturally¹². On the other hand, the release of HCN has been shown to protect bracken (*Pteridium aquilinum*)¹³ and cassava (*Manihot esculenta*)¹⁴ from insect attack. We have shown that *Sorghum* plants are best protected from insect attack when very young, and this protection is conferred by the HCN release on biting rather than by the cyanoglycoside itself or other products of its hydrolysis. Our work emphasises also

Table 1 Tests with hydrocyanic acid solution

Approx. concentration of HCN solution	No. of insects tested	Behaviour of 5th instar <i>Locusta</i> nymphs		
		No response	Feeding stops, but no backward movement	Feeding stops, also backward movement
1.0 M	5	0	0	5
0.1 M	5	0	0	5
0.01 M	10	0	4	6
1.0 mM	10	1	7	2
0.1 mM	10	4	6	0
0.01 mM	10	6	4	0
Water	10	10	0	0
Air	20	20	0	0

Different concentrations of hydrocyanic acid were introduced into the cibarial cavity of 5th instar nymphs through a chronically implanted cannula. Each insect had been deprived of food overnight and was tested during feeding on a palatable blade of grass. All tests were done during the first 3-min of feeding.

the importance of the initial rate of release of HCN when the insect bites.

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New approach to pollen culture

RESEARCH on pollen and anther culture is important for genetic and mutation studies, and has many potential applications in plant breeding and crop improvement. In our experience, the technique of pollen culture developed by Nitsch and co-workers^{1,2}, though successful with a wide range of species³, is much more difficult than anther culture and involves procedures which are unreliable and inefficient. Furthermore, plant yields are generally lower than when anther culture is used. We describe here an alternative approach which retains the simplicity and reliability of anther culture while eliminating the need for either mechanical disruption^{1,2} or surgical manipulation⁴ of anthers.

Our approach exploits the fact that, in many species, anthers dehiscence soon after inoculation, and if they are floated on shallow layers of liquid medium in plastic Petri dishes, pollen is 'shed' into the medium. This shed pollen will continue to grow if the anthers are removed from the dishes. Shedding continues throughout the culture period; hence, by frequent transfer of anthers to fresh medium a sequence of pollen cultures can be obtained from the same batch of anthers.

Examples of cultures of pollen shed from anthers, instead of mechanically isolated from them, are shown in Fig. 1 for *Nicotiana tabacum*. Illustrated is a series of cultures initiated with anthers of increasing age (Fig. 1 a–d) and in which the anthers have been transferred into fresh medium at 6, 10 and 14 d; in each case dishes were resealed after transfer of the anthers and reincubated together with a fourth dish containing the residual anthers (R). In cultures initiated at the unicellular pollen stage (microspores) (Fig. 1a), anthers do not open to any great extent and little shed pollen develops over the first 14 d. With increase in anther age, however, the time to anther opening decreases whereas embryo yields increase, and shed fractions develop at progressively earlier stages of culture (Fig. 1b, c). With anthers containing wholly young bicellular pollen grains (microgametophytes) (Fig. 1c), shed fractions develop after 0–6 d. Pollen is shed most rapidly in cultures of still older anthers (Fig. 1d) but as is now well known the embryogenic potential of older anthers is limited.

Chilling of buds before excision and culture of the anthers¹ is another important requirement for early dehiscence of tobacco anthers. We find periods of 12 d and longer at 7–8 °C optimal for this species. To minimise water loss during cold treatment, we store the buds in polythene bags wrapped in thin aluminium foil, a procedure which also maintains a high percentage of viable pollen (as assessed by fluorescein diacetate⁵). Illumination of cultures for the whole of the culture period has

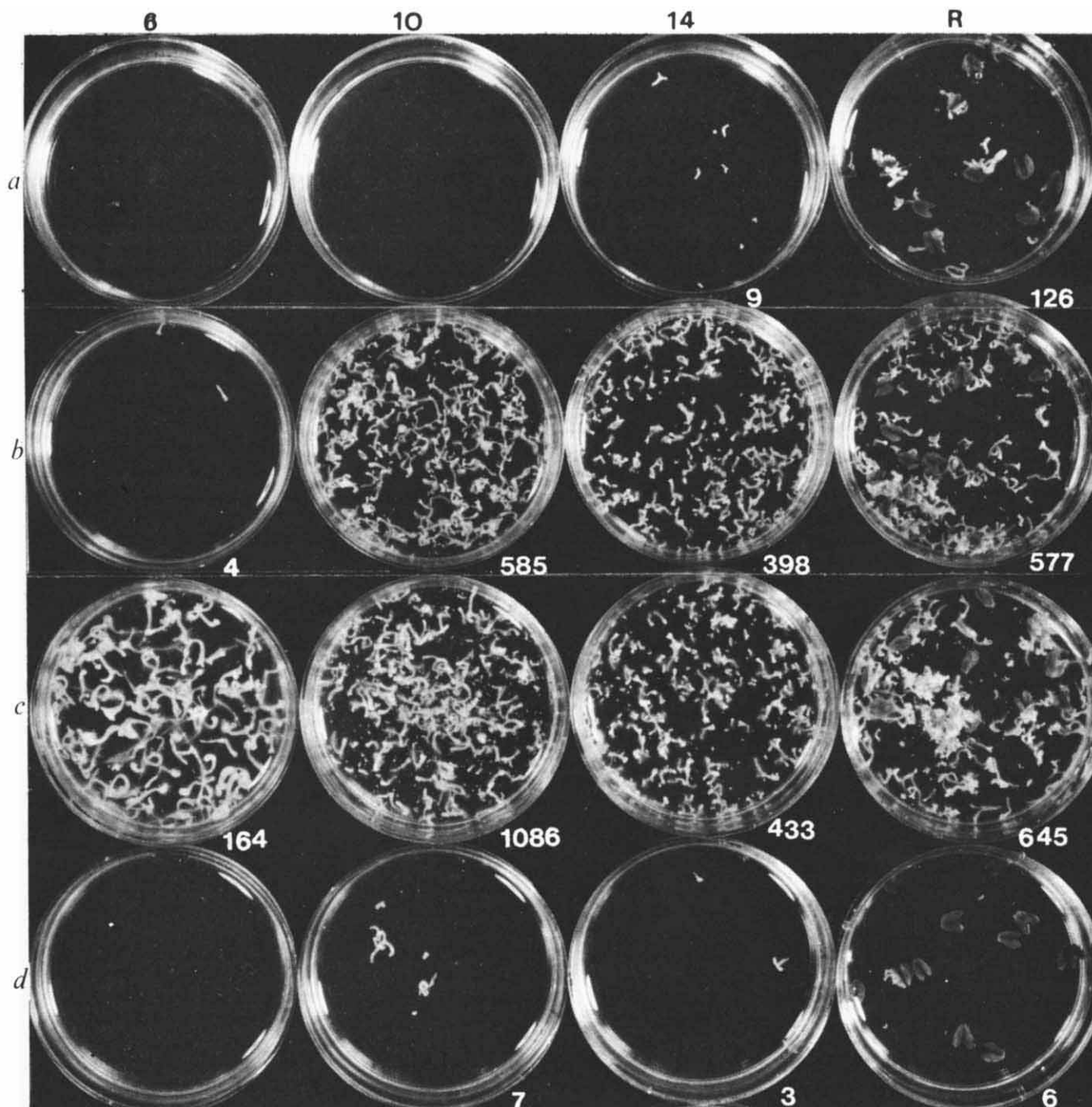


Fig. 1 *Nicotiana tabacum* cv. White Burley. Effect of developmental stage on anther dehiscence and liberation of pollen into liquid culture medium. *a*, Unicellular pollen stage (corolla length 13–15 mm). *b*, First pollen division stage (17–19 mm). *c*, Early bicellular pollen stage (21–23 mm). *d*, Bicellular stage just before deposition of starch (28–30 mm). In all cases, buds were stored at 7–8 °C for 12 d before excision and inoculation of the anthers. At inoculation, 12 anthers from three buds of similar stage were floated on the surface of 5 ml of liquid A medium. They were transferred to fresh A medium after 6, 10 and 14 d. All dishes were sealed with Parafilm and incubated at 28 °C in darkness for the first 14 d. After 14 d, they were transferred to light (Grolux tubes, 12-h day, 500 lx) at 25 °C. Embryo counts were made under a binocular after 35 d while the embryos were still small and separate from each other. Chromosome counts made on 10 embryos from each dish in (*b*) and (*c*) were all haploid. R, dish containing the residual anthers. A medium, major salts (half strength) and Fe-EDTA (full strength) of Murashige and Skoog¹⁰ plus sucrose 2%; pH 5.5.

so far proved detrimental as has shaking them on a horizontal shaker at 130 r.p.m. Our best results have been obtained with stationary cultures incubated in darkness for 14 d at 28 °C before transfer to light at 25 °C.

The performance of tobacco pollen 'shed' from anthers initially at the mitotic to early bicellular stages and that of pollen mechanically isolated from the anthers is indicated by the data of Table 1. Embryo yields are given for fractions shed into a simple nutrient medium (A medium) and fractions of isolated pollen mounted in either A medium or A medium supplemented with glutamine, serine and *myo*-inositol (AGSI

medium) as recommended by Nitsch². If we consider the data for the 8-d chilled material (which show the highest yields), it will be seen that the isolated pollen responds to AGSI medium (but not A medium) after preculture of the anthers for 3 d or longer, thus confirming Nitsch's findings. But in none of the pollen isolates is the yield as great as in the anther-culture controls. Shed fractions start to develop in the 8-d chilled material after 7 d of culture and by 9 d the yield exceeds that in any of the isolated fractions. The pollen is shed into A medium and the fact that it will then grow in the absence of the anther tissues implies a conditioning of the medium by the

Table 1 Comparison of embryo yields in cultures of anthers, isolated pollen and shed pollen of *Nicotiana tabacum* cv. White Burley

Preculture before isolation of pollen (d)												No isolation anther-culture controls	
Pollen fraction	Time of chill (d)	1		3		5		7		9		35	
		I	II	I	II	I	II	I	II	I	II	I	II
Isolated	0	0	0	0	0	0	0	0	3	1	5	22	35
	4	0	0	0	3	0	9	0	0	0	30	37	68
	8	0	0	0	71	0	35	2	34	2	63	120	116
Shed	0	0		0		0		0		1			
	4	0		0		0		0		8			
	8	0		0		0		34		90			

Mean embryo yields per anther from two experiments. I, Pollen cultured in A medium (see Fig. 1). II, Pollen cultured in AGSI medium (A medium plus L-glutamine 800 mg⁻¹, L-serine 100 mg⁻¹ and myo-inositol 5,000 mg⁻¹). In each experiment, three groups of 25 buds were harvested at the mitotic to early bicellular pollen stage (corolla length 17–23 mm). One group was dissected without cold treatment, the other two groups were stored at 7–8 °C for 4 and 8 d respectively before dissection. After dissection the five anthers from each bud were distributed singly into 5-cm Petri dishes containing 5 ml of liquid A medium, to give five replicates each of 25 anthers. One anther was then removed from each dish and floated on 5 ml of A medium, and a second anther from each dish was floated on AGSI medium (anther-culture controls). At 1, 3, 5, 7 and 9 d, one dish was taken from each group of five. Dead anthers (dark brown) were rejected; the remainder (about 20) were then removed and the dish resealed ('shed' fraction). The anthers removed were gently ground in a Potter glass homogeniser. Homogenates were filtered through 100-µm nylon and the filtrate centrifuged for 4 min at 100g. The supernatant was discarded. The pellet was resuspended in 10 ml of A medium, divided into two aliquots, recentrifuged and the supernatants again discarded. One pellet was inoculated into 5 ml A medium, the other into 5 ml of AGSI medium (isolated pollen). All dishes were sealed with Parafilm and incubated at 28 °C in darkness for the first 14 d, thereafter at 25 °C in Gro-lux light (12-h day, 500 lx). Embryos counted after 35 d. All buds were harvested within 2 weeks from the same batch of glasshouse plants grown through November to January under supplementary illumination.

anther tissues. Tapetum has long been thought to have a nutritive function and it is thus possible that conditioning of the medium stems from materials released from degenerating tapetal cells. Conditioning is a continuous process, and it is evident from the serial cultures of Fig. 1 that contact between anthers and medium for as little as 4 d is sufficient to support growth of large numbers of embryos. Evidence of medium-conditioning by anther tissues has also come from work on pollen isolated by surgical manipulation of anthers⁴.

In its simplicity the new approach to pollen culture has decided advantages for both applied and fundamental research. For mutation studies, we see no intrinsic objection to the use of fractions shed after between 0 and 6 d instead of fractions mechanically isolated from anthers at, say, 3–4 d. Besides eliminating possible deleterious effects of the homogenised anther tissues, the new procedure leaves the tissues intact, making them available for further experimentation and analysis. The new procedure is particularly attractive to large-scale manipulation of species possessing minute anthers, as in some of the cereals, and we have already found that, in barley, pollen calluses emerging from floating anthers are shed into the medium. Encouraging results have also been obtained in other Solanaceous species. Scrupulous attention must be given to anther-staging. For instance, in *N. knightiana*, anthers in the immediate post-first-division stage of the pollen are crucial, whereas in *Hyoscyamus niger*, the immediate pre-division stage seems to be the most productive. The success of the new procedure is undoubtedly due to the use of liquid, instead of agar, media. Advantages of liquid media, first reported for *N. tabacum* by M. Devreux (at the 1974 Haploid Conference held in Guelph), are now being realised by other workers and exploited in different species^{6–9}.

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Reproductive roles in the simultaneous hermaphrodite *Aplysia dactylomela*

THE opisthobranch gastropod *Aplysia* is a simultaneous hermaphrodite with internal fertilisation. The functional morphology of the reproductive system^{1–4} has been well studied and oviposition is known to be under some hormonal control^{5–7}. Adults may assume the role of sperm donor or recipient, or both at the same time. Recent discussions of the evolutionary significance of simultaneous hermaphroditism^{2,8–14} as well as considerations of the relative roles of sperm donors and recipients in selecting partners^{15,16}, have suggested that *Aplysia* offers an opportunity to investigate the processes involved in the assumption of different sex roles in the same animal. Accordingly we analysed data collected several years ago during other field studies of *Aplysia dactylomela*^{17,18}. We found that most animals were as likely to be sperm donors as recipients during different copulations, and there was a significant relationship between frequency of copulation and number of different partners in the total population studied. A few individuals, however, were found to copulate with fewer partners than sperm recipients.

We used *A. dactylomela* maintained in the Lerner Marine Laboratory, Bimini, Bahamas. Animals were collected during the day from the thalassia flats of the Bimini lagoon and placed in 1,000-l concrete tanks supplied with water from the ocean. The behaviour of groups of 5–10 animals was observed every 30 min between 0530 and 1130 and between 1200 and 2300 for 3 weeks; each group was observed 3–22 times. Individuals were identified by a colour-coded stainless steel safety pin inserted through the parapodium. Time of day had been shown to influence the probability that the animals would be found alone or copulating in their natural habitat¹⁸; we found that the same was true of animals maintained in laboratory conditions. Copulations occurred significantly more often in the morning ($P < 0.05$, χ^2 test¹⁹). We also confirmed that time of day did not significantly relate to the number of times animals were found alone, or in contact.

As Fig. 1 shows, most individuals were observed copulating

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between three and seven times. To facilitate statistical analysis of the roles assumed in copulation, we selected animals which copulated five times or more. This enabled us to use the binomial test with an associated probability of 0.06 or less¹⁹. By this criterion, we had 26 individuals which copulated five or more times. Of these, 17 did not copulate consistently in one or the other role, four were sperm recipients more often than donors, and five were sperm donors more often than recipients.

Thus our sample behaved as might be expected of simultaneous hermaphrodites in that the probability of finding individuals which consistently assumed one or another sexual role was relatively low. But we wanted to know whether the nine consistent animals represented a true behavioural subsample. It seemed that the three behavioural 'types' might be distributed differentially with respect to frequency of copulation.

As Fig. 2 shows, the number of times an individual copulated was positively correlated with the number of different partners ($r_s = 0.86$, $P < 0.01$). Animals which consistently assumed the same sex role were in a different part of the distribution from the others. The group medians for number of different partners (3.5) and number of observed copulations (5.5) segregated seven of these nine animals in the upper right quadrant of the graph. Thus all the consistent sperm recipients and three of the five consistent sperm donors copulated frequently and with many different partners. Further comparisons suggested that sperm recipients copulate with more partners than do sperm donors (Mann-Whitney U test, $U = 3$, $P = 0.056$).

This latter finding implies some degree of interindividual organisation that may be significant for adaptations of the species. We cannot eliminate the possibility that the differences in donor or recipient roles are due to sampling error; although individuals were collected at the same time, they may have been at different developmental stages or in different reproductive states. But if some part of any population behaves consistently as donors or recipients for a significant time, selection could act on them when pressures favour or disfavour behaviourally dimorphic individuals in reproduction.

We saw egg-laying animals simultaneously acting as sperm recipients but not as sperm donors. Genetic variability might therefore be increased in the least 'expensive' way if the sperm donor spends energy searching for sperm recipients, while the recipient spends energy on oviposition. Even though the whole population 'spends' equally on female and male functions, the differential behaviour patterns of a small group of individuals may well be significant for selection processes^{9,20}. Thompson and Bebbington⁴ suggested that *Aplysia* sperm are activated only after transfer to the recipient. Further work is needed to determine which sperm are more likely to fertilise the eggs—the more recently donated sperm, or that from previous copulations which has been stored for some time. Competition between sperm, such as that described for insects²¹, may be a mechanism in *Aplysia* by which more successful reproductive behaviours are selected.

Charnov and Bull¹¹ argued that in sessile simultaneous hermaphrodites selection favours the ability to alter the ratio

Fig. 1 Copulation frequencies observed in 37 individual *Aplysia dactylomela* maintained in tanks in the Lerner Marine Laboratory.

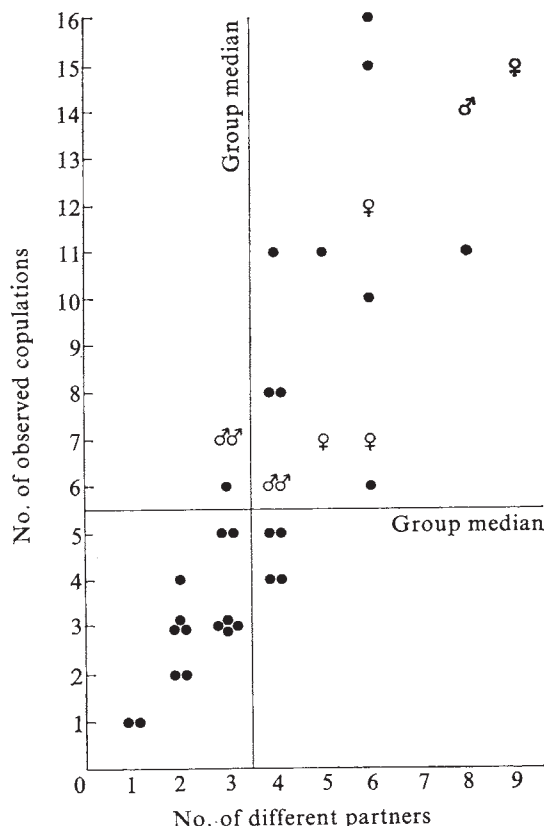
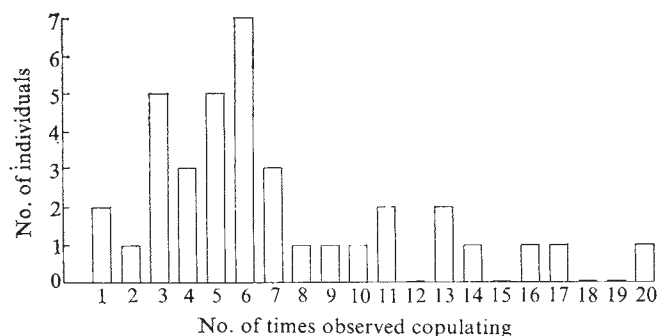


Fig. 2 Relationship between frequency of observed copulation and number of partners in *A. dactylomela*. ♀, sperm recipient; ♂, sperm donor; ●, one animal.

of sperm to ova in response to changing environmental conditions. It is possible that in the highly mobile *Aplysia*, the quantity and function of stored sperm may be regulated by the organisation of copulatory interactions.

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Gametogenesis in culture by gametocytes of *Plasmodium falciparum*

CONTINUOUS culture of human malaria parasites, *Plasmodium falciparum*, in human red blood cells was first reported by Trager and Jensen¹ and subsequently by Haynes and co-workers². The culture system described by Trager and Jensen supports the asexual multiplication of *P. falciparum* and the formation of gametocytes (the precursors of the gametes). Gamete formation by cultured *P. falciparum*, without which the parasites are unable to infect mosquitoes, has not been previously described. We report here that gametocytes of *P. falciparum* grown from parasites maintained by continuous culture *in vitro* are able to develop to the point at which they can be stimulated to undergo gametogenesis (exflagellation).

The isolate of *P. falciparum* used in this study was adapted to culture on 4 January 1977 from the blood of an individual who had become infected during a visit to Zaire from September to December 1976. This isolate, designated the Z strain, was obtained with the kind cooperation of Dr David Wyler. The parasites were adapted to growth in red cells of blood type A. Infected cells were suspended in culture medium at a haematocrit of 6%, placed in 30 ml tissue culture flasks (Corning no. 25100) with a 25 cm² culture surface area and incubated at 37 °C. Each flask contained 5.0 ml of cell culture. The medium used in routine culture was that described by Trager and Jensen¹ and consisted of RPMI 1640 powdered medium supplemented with HEPES buffer (25 mM) and 0.2% NaHCO₃ with 10% human serum type AB. Each day the culture medium was removed from the surface of the settled cells and replaced with fresh medium. Flasks were gassed with a mixture containing 6% O₂, 3% CO₂ and 91% N₂. During routine maintenance the parasite cultures were diluted with fresh red cells every 4–8 d. Parasite densities usually rose to between 1% and 2% of blood cells infected.

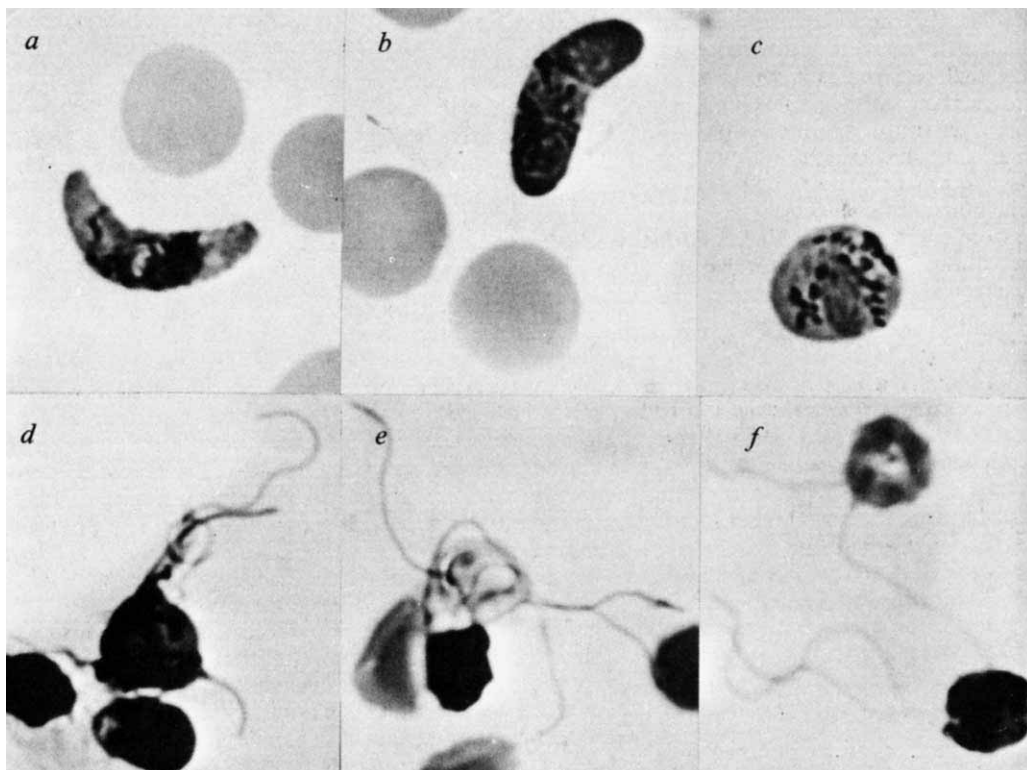
Smalley³ has reported that gametocytes in non-growing

cultures of *P. falciparum* require 10 d to develop from a merozoite to a stage approaching morphological maturity. In the present study certain cultures were maintained for 2–3 weeks without dilution to allow gametocytes to reach full maturity. In cultures maintained in this way immature gametocytes were usually present after 1 week. By 2 weeks numerous gametocytes were usually present including many with the morphological appearance of maturity (Fig. 1).

In one culture vessel, set up on 25 February 1977 when the parasites had been maintained in continuous culture for almost 7 weeks, the regular medium was replaced 15 d later with one containing 50% serum instead of the usual 10%. The culture was maintained in this medium thereafter. Eight days after substituting with the new medium the gametocytes density was 0.6%. At this stage almost 50% of the gametocytes were apparently mature by morphological criteria on blood smears stained with Giesma's stain. Mature gametocytes of both sexes were crescent shaped cells with rounded ends and pigment granules concentrated near or over the nucleus near the centre of the crescent (Fig. 1). Male and female gametocytes were distinguished from one another by their staining properties. The cytoplasm of female gametocytes stained blue while that of males stained orange-pink and was not easily distinguished from the nucleus. About 35% of all gametocytes were morphologically mature females and about 10% were morphologically mature males. The remaining gametocytes were in various stages of development.

Gametocytes from this culture were stimulated to undergo gametogenesis by the following method. On the eighth day after substituting with medium containing 50% serum the cells were evenly resuspended in their own culture medium and 0.3 ml of the suspension dispensed in a serum tube into 3.0 ml of foetal bovine serum (FBS) adjusted to pH 8 with 1.5% NaHCO₃. The cells were sedimented by centrifugation for 30 s; most of the supernatant was removed and the packed cells were resuspended in a minimum volume of the remaining supernatant fluid. One drop of this cell suspension was placed on a microscope slide, a cover slip placed on top and the preparation examined

Fig. 1 Gametocytes and gametogenesis of *P. falciparum* in culture. *a*, Morphologically mature macrogametocyte; *b*, morphologically mature microgametocyte; *c*, macrogamete 25 min after initiation of gametogenesis; *d*, *e*, *f*, exflagellating microgametocytes showing the release of microgametes 25 min after initiating gametogenesis. All preparations were methanol fixed and stained with Giesma's stain.



under a microscope. Between 5 and 10 min after the cells had been resuspended in FBS extensive rounding of gametocytes was observed. At 15 min the first exflagellation was seen (Fig. 1). During subsequent observation, up to 45 min, about one exflagellating male gametocyte was observed per 10,000 red blood cells. This represents about one exflagellating male gametocyte for every five male gametocytes judged to be mature by morphological criteria. Over 50% of female gametocytes judged morphologically mature were found to have rounded up to form female gametocytes when examined on blood smears stained with Giesma's stain.

When gametocytes were examined on a slide in culture medium without washing in FBS at pH 8.2, no evidence of gametogenesis was seen during 45 min of observation under the microscope.

Two days later the experiment was repeated on the same culture using human serum adjusted to pH 8.2 instead of FBS as the exflagellating medium. Gametogenesis (exflagellation) was again successfully initiated. The same method for stimulating exflagellation was applied to three other cultures. These were only 2 weeks old and had been maintained throughout in medium containing 10% human serum. In two of these cultures exflagellation was successfully initiated although the frequency of exflagellation events was less than that observed in the 3-week-old culture.

It is clear from these results that gametocytes produced by the Trager-Jensen system of malaria cultures can develop to a point at which they are capable of undergoing gametogenesis. At this stage our observations are not adequate to define the precise conditions required to achieve this reproducibly. Prolonged maintenance of cultures for at least 2 weeks is probably important to allow gametocytes to reach full maturity. Transfer to a medium containing high serum may also be important in this respect. It is well known that certain malaria parasites (for example, rodent malarias) lose their ability to form gametocytes after prolonged blood passage in laboratory hosts without mosquito transmission. An analogous situation may apply to *P. falciparum* maintained for prolonged periods in culture. If this is true it may be significant that we achieved our results with a line of parasites recently isolated from a natural infection.

Account should also be taken of the fact that the demonstration of exflagellation *in vitro* depends on the use of appropriate methods for stimulating gametogenesis. In studies with other species of malaria parasites it has been found that bicarbonate-containing media at pH 8.0, most effectively animal sera, are excellent for this purpose^{4,5}. At pH values below 7.7 and in media from which bicarbonate is absent exflagellation usually does not take place. In the conditions used to culture *P. falciparum* the Trager-Jensen culture medium is maintained at a pH of 7.3 to 7.4. The presence of a powerful buffer (25 mM HEPES) in this medium may prevent the pH from rising sufficiently rapidly to initiate gametogenesis during equilibration with the atmosphere. Thus after exposing a drop of the cell suspension to the atmosphere, the method commonly used to initiate exflagellation, we were unable to observe gametogenesis by gametocytes in their original culture medium. By washing and resuspending cultured gametocytes in bicarbonate supplemented sera at pH 8.0, exflagellation was readily demonstrated.

Our observations demonstrate that cultured blood stages of *P. falciparum* are capable of generating gametocytes able to undergo gametogenesis. Although the conditions which would enable this to be carried out on a regular basis are not completely defined there is good reason to expect that cultured blood stages of *P. falciparum* may be used to generate the gametes and mosquito stages of this parasite.

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'Strong' phase response curve for the circadian rhythm of locomotor activity in a cockroach (*Nauphoeta cinerea*)

ANALYSIS of entrainment in circadian systems depends on a knowledge of the phase resetting effects of the light (or temperature) pulses perturbing the circadian oscillation. Thus when a light pulse of a particular intensity and/or duration falls at certain phases of the free-running oscillation it may generate phase-advances ($+\Delta\phi$) in subsequent activity; when it falls at other phases it may generate phase-delays ($-\Delta\phi$). A plot of such phase changes, both magnitude and sign, as a function of the phase of the oscillation so perturbed, is called a phase response curve (PRC) (refs 1, 2). In a systematic study of the resetting effects of light pulses on the rhythm of pupal eclosion in *Drosophila pseudoobscura*, Winfree³ demonstrated two topologically different types of PRC, depending on the strength of the signal. When light pulses were 'weak' (< 50 s blue light, $10 \mu\text{W cm}^{-2}$) phase shifts were small. When pulses were 'strong' (> 50 s) phase shifts became abruptly larger, up to 10–12 h. Because of the way Winfree's data were graphically presented, weak resetting curves were called type 1 and strong curves type 0. An abrupt change from type 1 to type 0 has been observed only in circadian systems which control physiological events such as pupal eclosion (refs 1–3 and D.S. in preparation) and oviposition⁵ in populations of insects. Rhythms of locomotor activity in single animals, on the other hand, have only shown low amplitude type 1 curves^{6,7}. Entrainment in 'physiological' rhythms is in most cases mediated by extraoptic photoreceptors and a hormonal output⁸. 'Behavioural' rhythms, on the other hand, most frequently involve entrainment by way of the organised photoreceptors^{9,10} and an electrical (neural) output^{10–12}. Truman used these differences to distinguish two types of biological clock¹³. Here, however, we report PRCs which switch from type 1 to type 0 in the locomotor activity rhythm of a cockroach *Nauphoeta cinerea*, which might from earlier literature have been expected to show only type 1 resetting.

Newly-emerged males of *N. cinerea* were housed singly in small Perspex running wheels, in light-tight boxes at $25 \pm 0.5^\circ\text{C}$. Each revolution of the running wheel caused four closures of a magnetic proximity switch, and the activity of the cockroach was thus recorded by an Esterline-Angus event recorder. For each insect, activity records for successive 24-h periods were pasted down in chronological order on poster boards (Fig. 1). Records of locomotor activity were then photographed and 'double-plotted' to enhance any rhythmic features.

Insects were initially entrained to a light cycle of 12 h of light and 12 h dark (LD 12:12) for 10–14 d. The lights were then turned off and the activity rhythms allowed to free-run in continuous darkness. Measurements of free-running periods (τ) were made by eye-fitting a line to the onsets of activity (Fig. 1), the slope of the line indicating τ . Cockroaches in free-running conditions were then perturbed by single 3 h or 12 h light pulses ($240 \mu\text{W cm}^{-2}$), with

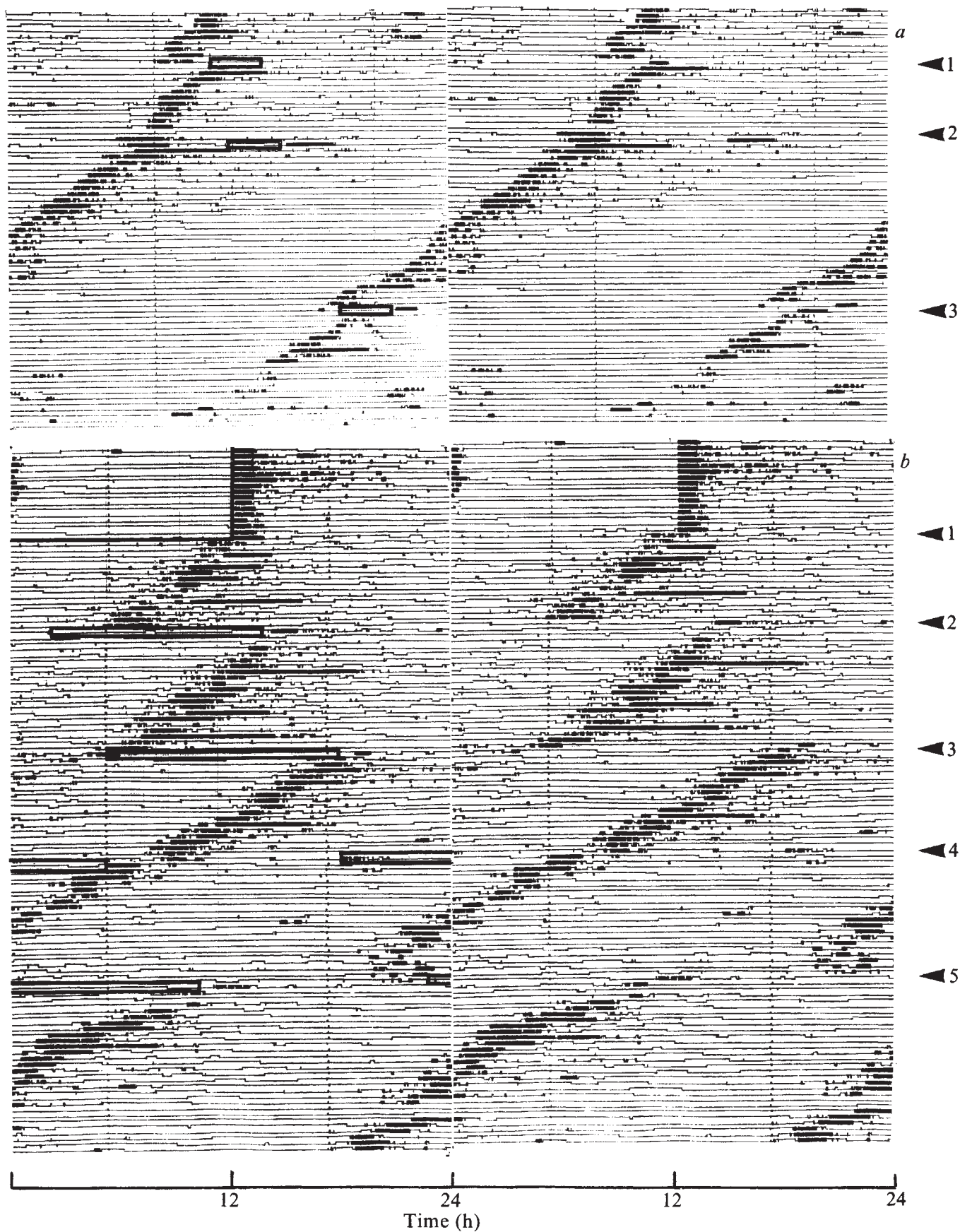


Fig. 1 Locomotor activity rhythms of two males of the cockroach *Nauphoeta cinerea*. *a*, 'Free-running' in continuous darkness (DD) and perturbed by single 3-h pulses of white light ($240 \mu\text{W cm}^{-2}$) at: 1, circadian time (Ct) 14.7 ($-\Delta\phi 2.5$ h); 2, Ct 17.1 ($+\Delta\phi 0.5$ h); 3, Ct 13.3 ($-\Delta\phi 3.1$ h). *b*, Initially entrained to LD 12:12, then: 1, 'free-running' in DD and perturbed by single 12-h pulses of white light ($240 \mu\text{W cm}^{-2}$) at: 2, Ct 10.2 ($-\Delta\phi 9.8$ h); 3, Ct 13.5 ($+\Delta\phi 11.8$ h); 4, Ct 22.3 ($+\Delta\phi 2.2$ h); 5, Ct 15.9 ($+\Delta\phi 7.0$ h). Activity records are 'double-plotted' to enhance rhythmic features. Light treatments are shown as open boxes in the left-hand panel. $-\Delta\phi$, delay phase shift (h); $+\Delta\phi$, advance phase shift (h).

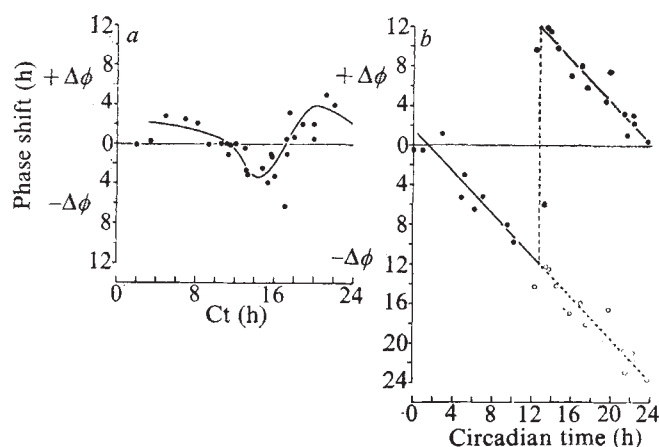


Fig. 2 Phase response curves for 3-h (a) and 12-h (b) pulses of white light ($240 \mu\text{W cm}^{-2}$) in the cockroach *Nauphoeta cinerea*. For 3-h pulses the response curve is of the 'weak' type 1 with small phase changes (4–5 h). For 12-h pulses the response curve is of the 'strong' type 0, drawn either as a 'saw-tooth' curve showing phase delays and phase advances (up to 12 h), or as a monotonic straight line with all phase changes plotted as delays ($y = -1.06572x + 1.6640$; $r = 0.96$; $P < 0.001$).

the resetting pulses being timed to occur at all phases (circadian times, Ct) of the insects' endogenous circadian period. Phase shifts, in hours, were measured as displacements in activity onsets before and after the pulse.

The phase response curve for 3-h pulses of white light ($240 \mu\text{W cm}^{-2}$) (Fig. 2a) was of the low 'amplitude' type 1 with phase delays early in the 'subjective night' (Ct 12–18) and phase advances late in the 'subjective night' (Ct 18–24). Maximum phase changes were in the order of 4–5 h. Phase advances were distinguished from phase delays, not only by their final steady state in relation to the activity onset before the pulse, but also on the criterion of reaching that steady state through a series of non-steady-state or transient cycles, which are evident for advances but not delays¹. For 12-h pulses phase changes were much larger, the resultant response curve (Fig. 2b) either being represented by a high amplitude saw-tooth with a 360° 'dataless' change of phase between delays and advances at about Ct 13 or, more realistically perhaps, by its linear or monotonic form, treating all phase changes as delays. In this connection all phase changes except one (that starting at Ct 2.9) were devoid of transients.

Earlier results with cockroaches (*Leucophaea maderae*) showed only small phase shifts even with light pulses of $2,000 \text{ lx}$ (about $800 \mu\text{W cm}^{-2}$) and 12 h duration¹⁴. But other results for the same species¹⁵ have demonstrated strong type 0 resetting with very intense light ($80,000 \text{ lx}$, about $32,000 \mu\text{W cm}^{-2}$) and about 12 h duration. The intensity of the light required to elicit a strong phase response curve in *L. maderae* is therefore about two orders of magnitude greater than in *N. cinerea*. Both of these studies, however, show that type 0 response curves can be obtained for 'behavioural rhythms' (presumably using compound eyes as photoreceptors, and having a neural output), provided that the energy of the perturbing signal is sufficiently high.

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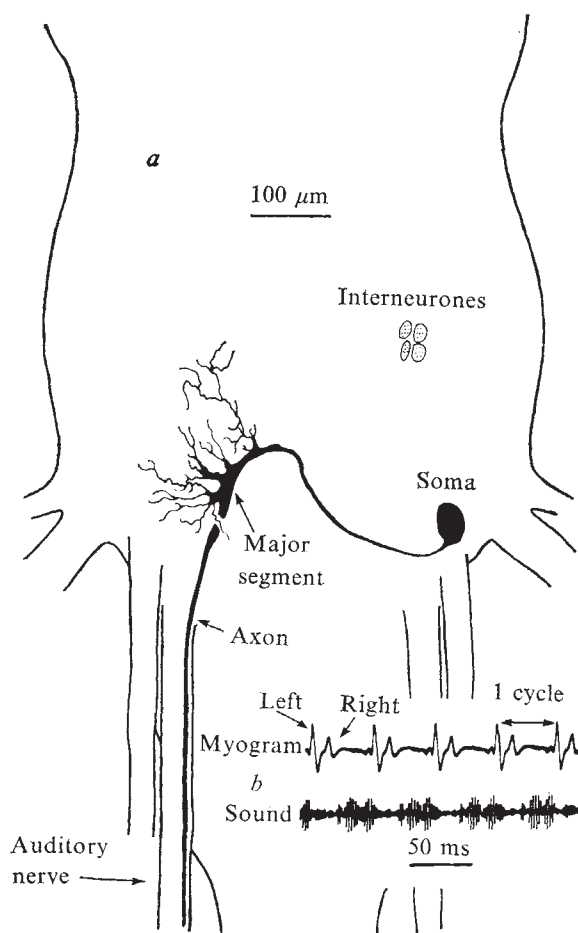
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Neuronal generation of singing in a cicada

CICADA song may be regarded as a simple rhythmical behaviour because it is highly stereotyped and involves regular spikes in a single pair of motoneurons. It was the subject of an early study of intracellular activity in central neurones¹. Here I show that the neuronal generator for song of the Australian bladder cicada, *Cystosoma saundersii* (Westw.) is continually active at twice the frequency of motoneurone spikes and, in common with other rhythmical behaviours in arthropods^{2–4}, involves non-spiking interneurons.

The song of a cicada is composed of a regular series of sound pulses, each pulse being produced by a snap-like collapse of a stiff ribbed structure, a tymbal^{5–8}. There is a tymbal on each side of the anterior of the abdomen. Collapse of a tymbal is caused by a twitch contraction of its tymbal muscle, and the inherent elasticity of the tymbal restores its original shape. Each tymbal muscle is in-

Fig. 1 a, Structure of a tymbal motoneurone, viewed dorsally. The abdominal and metathoracic ganglia of *C. saundersii* are fused, and their outline is drawn. The motoneurone was stained by back-filling with dilute (2%) CoCl_2 . Somata of four interneurons, which were consistently stained by this method—probably trans-synaptically¹⁰—are also shown. Most of the processes of the motoneurone, as well as its soma, lie near the dorsal surface of the ganglion. **b**, Calling song of *C. saundersii*—sound produced, and simultaneous myogram recorded by a pair of silver electrodes implanted in the left tymbal muscle.



nervated by a single motoneurone, one spike in which is sufficient, in *C. saundersii*, to elicit a twitch contraction of the muscle (*C. saundersii* has neurogenic tymbal muscles; some species have myogenic tymbal muscles⁶⁻⁸). Figure 1a shows the geometry of a tymbal motoneurone. The pattern of spikes in tymbal motoneurons during the 'calling' song of *C. saundersii* is shown in Fig. 1b; it is a rather unusual rhythmical activity in that there is a delay of one quarter of a cycle between the spike in one tymbal motoneurone and the spike in its contralateral partner. Either the left or the right tymbal motoneurone may lead and, following short pauses when one or both motoneurons stop spiking, the motoneurons often swap the lead. 'Protest' songs, which a cicada produces when disturbed or when electrical shocks are applied to its brain, are similar although usually of lower spike frequency.

Intracellular recordings from tymbal motoneurons reveal regular, smooth waves in membrane potential (Fig. 2a, b). These waves occur continually, whether or not the cicada is singing. Waves in the left and right motoneurons have the same frequency, which suggests that they originate within a shared oscillator mechanism, but are half a cycle out of phase (Fig. 2a). In recordings from different animals at varying times of day the frequency of the waves ranges from 55 to 80 Hz, although in any particular recording session (up to 2 h) waves have a constant frequency, with only 1 in 200 waves being slightly longer or shorter than usual.

Two sets of observations show that the waves originate in interneurons rather than in the motoneurons them-

Fig. 2 Intracellular recordings from tymbal motoneurons in a non-singing cicada. Recordings were made with strong glass electrodes filled with 2 M potassium acetate and with resistances 50–80 M Ω . The electrodes were driven through the intact sheath. In *a*, recordings are from somata; in other traces they are from unidentified processes. *a*, Waves recorded simultaneously from left (upper trace) and right (lower trace) tymbal motoneurons. *b*, Variation in wave amplitude immediately following a period of vigorous singing. *c*, Effects of passing current into a tymbal motoneurone. Current was passed through the recording electrode by means of a bridge circuit. *c*, Upper record, depolarising current elicits spikes on top of some waves. The spikes reached the tymbal muscle. Lower record, hyperpolarising current alters the shape but not the frequency of the waves. *d*, Antidromic spikes which follow, by 9 ms, shocks applied to the tymbal muscle, do not effect the timing or the amplitude of the next wave. Arrow denotes antidromic spike. Calibrations—*a*, 4 mV, 40 ms; *b*, 4 mV, 200 ms; *c*, 40 mV, 40 ms; *d*, 20 mV, 20 ms.

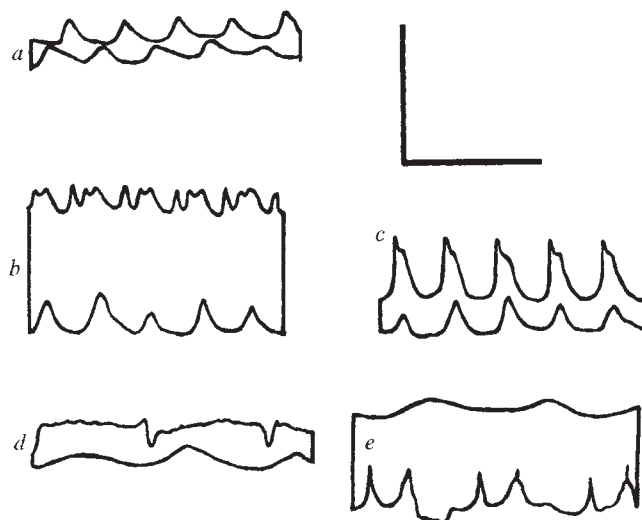
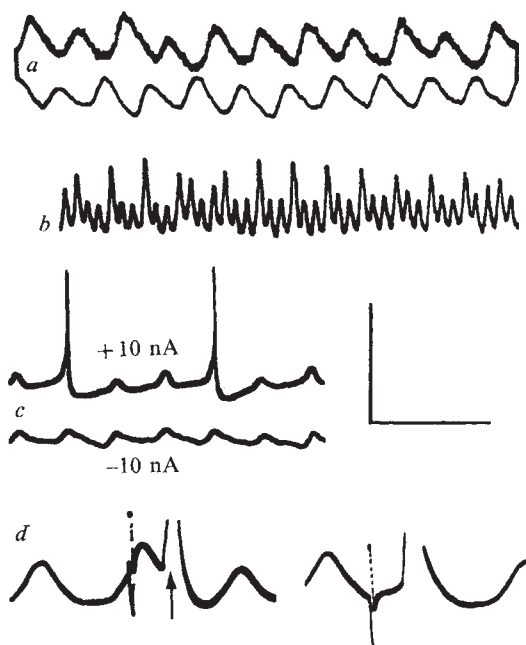


Fig. 3 Recordings of phase-locked potentials in tymbal motoneurons and presumed interneurons of non-singing cicadas. *a*, Intracellular recordings from a tymbal motoneurone (upper trace) and from a non-spiking interneurone (lower trace). *b*, Intracellular recordings from a tymbal motoneurone (lower) and a non-spiking interneurone (upper). Wave frequency, 65 Hz. *c*, Hyperpolarising (-10 nA) the interneurone of *b* alters its waveform and reduces the frequency of waves in it and in the motoneurone to 64.5 Hz. *d*, An interneurone (upper trace) which spiked once during each wave in a tymbal motoneurone (lower). The neurone is presumed to be an interneurone because no spikes in the motor nerves could be correlated with the frequency of waves in the tymbal motoneurons. *e*, An interneurone (lower) which spiked twice during each wave in a tymbal motoneurone (upper). Calibrations—*a*, motoneurone 10 mV, interneurone 1 mV, 40 ms; *b*, *c*, interneurone 2 mV, motoneurone 10 mV, 40 ms; *d*, interneurone 2 mV (a.c., extracellular), motoneurone 20 mV, 20 ms; *e*, motoneurone 5 mV, interneurone 1 mV, 20 ms.

selves. First, depolarising or hyperpolarising a motoneurone does not alter the frequency of the waves, but can affect their shape (Fig. 2c). Second, an antidromic spike in a tymbal motoneurone does not affect the timing or the amplitude of subsequent waves (Fig. 2d). The motoneurons do not seem to be directly coupled by an electrical or a chemical synapse because an antidromic or orthodromic spike in one tymbal motoneurone produces no recognisable effect on its partner. Each motoneurone receives inputs from different interneurons because there is no significant correlation between the amplitude of a wave in one motoneurone and the amplitude of the next wave in its partner. In the recording from which Fig. 2a is taken, for example, the correlation coefficient for amplitudes of sequential waves in the left and right tymbal motoneurons, calculated by a method of least squares, is 0.275, which is not significant.

Two observations strongly suggest that non-spiking interneurons drive the waves in the motoneurons. First, the waves are very smooth—discrete post-synaptic potentials have never been seen in them. Second, intracellular recordings have been made from a number of non-spiking interneurons with activity phase-locked to the waves in the motoneurons (Fig. 3a, b). The evidence that these neurones were non-spiking interneurons is that neither direct, strong depolarisation nor stimulation of motor axons elicited spikes in them. It is likely that these interneurons are involved in singing because the frequency of the waves in the tymbal motoneurons, which is twice that of spikes during song, is not related to that of any other rhythmical behaviour, such as ventilation, flight or walking. Unfortunately, passing current into an interneurone rarely affected the frequency of the waves, either in the interneurone or in a motoneurone. One exception is shown in Fig. 3b, c, where hyperpolarising the inter-

neurone caused a small but reversible decrease (65–64.5 Hz) in the frequency of waves in both the interneurone and the motoneurone and a change in the waveform in the interneurone. Possibly several interneurons are involved in generating the song rhythm and communicating it to the motoneurons, and passing current into only one interneurone has little effect on the rhythm generator. In addition to the non-spiking interneurons several interneurons which produce spikes phase-locked to the waves in the motoneurons were found (Fig. 3*d, e*).

When a *C. saundersii* sings each tymbal motoneurone produces a spike on every second wave (Fig. 4*a*). The frequency of tymbal motoneurone spikes and of sound pulses generated by each tymbal is therefore half that of the waves in the tymbal motoneurons. Because the waves in the left and right motoneurons are exactly out of phase, spikes in them are separated by one quarter of a cycle. Often, in 'protest' songs, spikes occur less regularly than every second potential (Fig. 4*b, c*)—the rule seems to be that spikes never occur on sequential waves.

The reason for this limit to spike frequency is unclear, but there are three observations which may indicate relevant factors. First, some waves which do not culminate in a spike reach more positive potentials than the take-off potential for spikes (Fig. 4*c*). Bearing in mind that recordings were probably never made from the spike initiation region of a tymbal motoneurone, the simplest explanation for this is that interneurons other than those

involved in producing the waves in a non-singing cicada are involved in producing spikes. Second, each of the largest waves is often followed by a much smaller one (for example, Fig. 2*b*). This is not due to direct negative feedback from a motoneurone because an antidromic spike does not affect the amplitude of subsequent waves and there is no correlation between the amplitude of one wave and the amplitude of the wave following it in the same motoneurone (range of serial correlation coefficients for 15 recordings: 0.04 to 0.43). Third, bursts of spikes in a tymbal motoneurone were never seen and even depolarising currents as great as 200 nA injected into a tymbal motoneurone soma produced only single spikes.

It may be a general feature of rhythmical behaviours that their neuronal rhythm generators are continually active. In quiescent locusts the neuronal oscillator for flight produces subthreshold depolarisations at flight frequency in motoneurons⁹. To use a mechanical analogy, nervous systems may well use 'clutches for engaging gear' rather than 'starter motors' in producing rhythmical movements.

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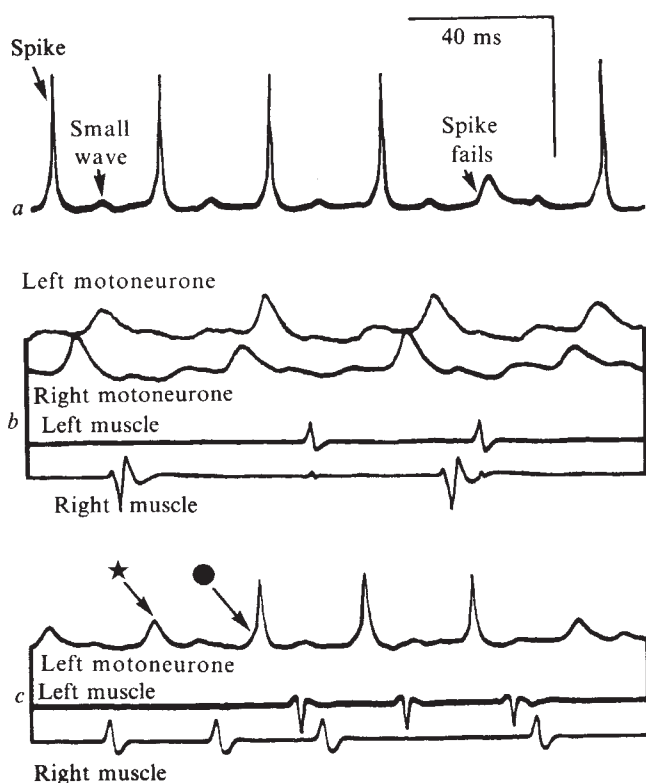
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Fig. 4 Intracellular recordings from tymbal motoneurons when a *C. saundersii* is induced to sing by stimulating its brain electrically. *a*, Vigorous singing—spikes alternate with small waves. One large wave, where the spike fails, reaches a more positive potential than the take-off potential for spikes. *b*, Potentials recorded simultaneously from left and right somata. Spikes, identifiable by their sharp rising phase and from the muscle recordings, are rather infrequent, occurring at most on every third wave (left motoneurone). Note that not every large wave gives rise to a spike. *c*, The electrode for this recording had penetrated the ganglion near the origin of the auditory nerve (see Fig. 1). ★, Some waves reach a more positive potential than ●, the take-off potential for spikes. Calibrations—*a*, 40 mV, 40 ms; *b, c*, 20 mV, 40 ms.



Site of *pcd* gene action and Purkinje cell mosaicism in cerebella of chimaeric mice

PURKINJE cell degeneration, *pcd*, is a new autosomal recessive mutation in mice. Affected animals are characterised by a moderate ataxia that results from the postnatal loss of virtually all cerebellar Purkinje cells¹. We describe here experiments to determine whether the *pcd* gene acts intrinsically within the Purkinje cell or whether the degeneration is secondary to a primary lesion in some other cell type. In this study, experimental chimaeras were made which contained mixtures of mutant (*pcd/pcd*) and genotypically normal (+/+) cells (these chimaeras are denoted *pcd/pcd* ↔ +/+). The results indicate that the *pcd* gene does act within the Purkinje cell. The chimaeras have also been used to examine the surviving population of +/+ Purkinje cells for evidence of clonal development. Surprisingly, these preliminary studies reveal no evidence of clones; the distribution of cells derived from the +/+ component seems random.

The *pcd/pcd* ↔ +/+ chimaeras were produced by Tarkowski² and Mintz³ techniques of aggregation of eight-cell embryos as described previously⁴. Since *pcd/pcd* males are sterile, embryos were obtained from *pcd/pcd* females that were mated to +/*pcd* males so only 50% of the chimaeras were expected to be homozygous for *pcd*. Five chimaeras were produced. One female chimaera was proven to be homozygous for *pcd* by progeny testing. In addition, two male chimaeras were also homozygous for *pcd* as evidenced by their abnormal sperm (a pleiotropic effect of the *pcd* mutation¹) and loss of cerebellar Purkinje cells. All three *pcd/pcd* ↔ +/+ chimaeras appeared behaviourally normal. They were not, however, subjected to any special behavioural testing.

In all three chimaeras the cerebella were mosaics, for it was obvious in histological sections that many Purkinje cells had degenerated. Although the gene was expressed in the chimaeras,

that is not evidence that it acts within the Purkinje cells. If the *pcd* locus exerts its primary effect within the Purkinje cell and is not cured by the presence of normal cells, then in the chimaera the cells that are *pcd/pcd* will degenerate and the surviving cells will be the $+/+$ cells. Alternatively, if the degeneration of Purkinje cells is a secondary effect of the *pcd* locus acting in another cell type, then the surviving cells would be a mixture of *pcd/pcd* and $+/+$ cells. To determine the genotypes of the surviving cells, it is necessary to use an independent cell marker.

One of the *pcd* chimaeras was also chimaeric for the structural gene for β -glucuronidase. This enzyme has been used previously as a cell marker in other tissues^{5,6}. In the *pcd* chimaera, the *pcd/pcd* cells were homozygous for *Gus^b*, the high enzyme activity allele, whereas the $+/+$ cells were homozygous for *Gus^h*, the low activity allele. This chimaera is denoted *pcd/pcd Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h*. A new, more sensitive histochemical procedure developed by Feder⁶ was used. This procedure is limited in its use as a central nervous system (CNS) cell marker, but it can be used to determine the genotype of Purkinje cells (R. J. M. and R. L. Sidman, unpublished). In *Gus^b/Gus^b* non-chimaeric controls, all of the Purkinje cells show enzyme activity as evidenced by the red reaction product. In *Gus^h/Gus^h* non-chimaeric controls, none of the Purkinje cells has sufficient enzyme activity to produce any noticeable staining. In *Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h* chimaeras (both components being $+/+$ at the *pcd* locus), a mosaic of stained and unstained Purkinje cells is observed (Fig. 1a). Virtually all Purkinje cells can be assigned to one class or the other. When sections from the *pcd/pcd Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h* chimaera were stained by this technique, none of the surviving Purkinje cells showed enzyme activity (Fig. 1b), indicating that they were *Gus^h/Gus^h* and therefore $+/+$ at the *pcd* locus. Thus, in the chimaera, the *pcd/pcd* Purkinje cells have degenerated leaving the genetically normal $+/+$ cells.

The simplest interpretation of this is that the genic alteration

Fig. 1 Sagittal sections (7 μ m) of cerebellar cortex stained histochemically for β -glucuronidase and counterstained with methyl green. The mice were perfused with cold (0–4 °C) 4% formaldehyde in phosphate buffer. The brains were processed and stained as described by Feder⁶. The sections were stained for 3 d at 37 °C with fresh substrate added daily. In these photomicrographs the red precipitate at the sites of glucuronidase activity seems dark gray. **a**, Cerebellar cortex of a *Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h* chimaera. Some of the large Purkinje cells are stained (arrows with dots) indicating they are from the *Gus^b* component; others are not stained (arrows) and are, therefore, *Gus^h*. **b**, Cortex of a *pcd/pcd Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h* chimaera. None of the surviving Purkinje cells is stained (arrows) indicating they are from the $+/+$ *Gus^h/Gus^h* component. To the left is a gap left by degenerated Purkinje cells. In both **a** and **b** the other intense staining in the Purkinje cell layer is probably in Golgi epithelial cells (Bergmann glia). Scale bar, 20 μ m.

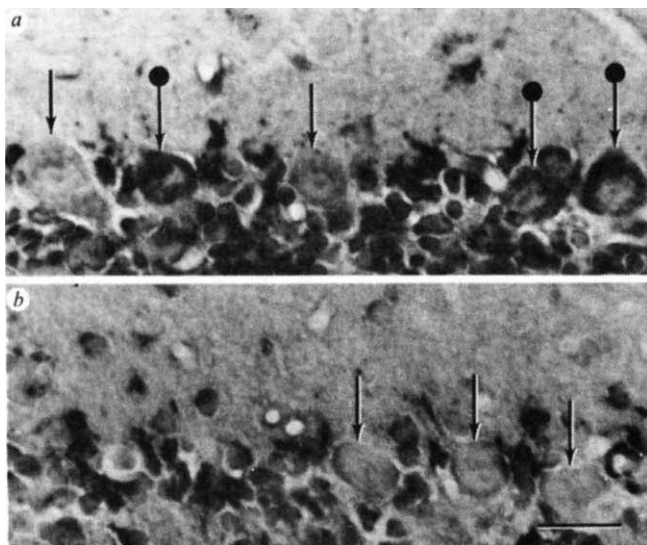
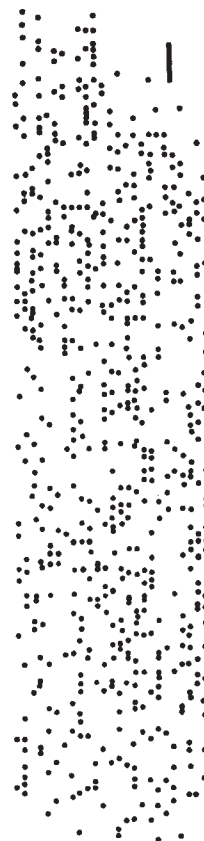


Fig. 2 Distribution of surviving $+/+$ Purkinje cells in a *pcd/pcd ↔ $+/+$* chimaera in which 75% of the cells have degenerated. With a drawing tube, the positions of Purkinje cells along the Purkinje cell layer of the lobulus centralis in 20- μ m sagittal sections were traced on tracing paper. The presence of nuclei and nucleoli were also marked to help eliminate cells present in adjacent sections. After aligning the tracings by superimposition, a map reader was used to measure distances along the Purkinje cell lamina. The positions of the cells were then plotted in straight lines on graph paper. Twenty-five serial sections (500 μ m) were recorded. The size of the dots is scaled to the average Purkinje cell diameter. Thus, the figure represents the location of cells as they would appear if the folium was flattened out and viewed from above. A two-dimensional reconstruction such as this might change the shape of a patch or clone of cells, but it would not disrupt it. Throughout the rest of the cerebellum of this chimaera, the proportion of cells that survived was relatively constant; there were no regions where the density of Purkinje cells was normal and no regions where they had all degenerated. Thus, the portion reconstructed is not at an interface between large patches of mutant and normal cells. Scale bar, 100 μ m.



at the *pcd* locus is affecting the Purkinje cell directly. This conclusion is supported by an ultrastructural study of *pcd/pcd* Purkinje cells in which a unique series of abnormalities in Purkinje cells was observed. Nothing was observed in this earlier study that would suggest the gene was acting anywhere else in the cerebellum other than the Purkinje cell⁷. One alternative is that the *pcd* locus is acting in some other cell, also of the *pcd/pcd* genotype, with which the Purkinje cell must interact. This seems highly unlikely, for at least some *pcd/pcd* Purkinje cells should have been close enough to $+/+$ cells of this 'other' cell type during development to be saved, but none was.

Since the surviving Purkinje cells in the chimaera are all of the same genotype, they had, at some time in development, common ancestors. Their distribution can be studied for evidence of clones that might be of developmental, anatomical and/or functional significance. In single sections the mosaicism is apparently extremely fine with some 'patches' being a single cell. This, however, must be cautiously interpreted, as what seems to be a single, isolated cell could be on the edge of a much larger patch or there could be narrow clones running perpendicular to the plane of section. The Purkinje cell layer is relatively well-suited for serial reconstruction for it is a single cell-thick lamina so the positions of cells can be plotted in two dimensions. A serial reconstruction showing the distribution of surviving Purkinje cells in a portion of the central lobule (lobulus centralis⁸) of one of the *pcd* chimaeras is shown in Fig. 2. There does not seem to be any pattern or evidence of clones; the distribution seems to be random.

Although it might seem unlikely, the random distribution could be the result of rearrangement of the $+/+$ cells after the *pcd/pcd* cells degenerate. This possibility was investigated by examining chimaeras in which there was no cell loss but in which the genotype of Purkinje cells was determined with the β -glucuronidase cell marking technique. Sections from a *Gus^b/Gus^b ↔ $+/+$ Gus^h/Gus^h* chimaera were analysed to estimate the number of cells in a clone of Purkinje cells. The estimate is based on a comparison of the mean patch size (contiguous

Table 1 Mean patch size and estimate of clone size for *Gus^b/Gus^b* Purkinje cells in a *Gus^b/Gus^b ↔ Gus^b/Gus^h* chimera.

Mean no. of <i>Gus^b/Gus^b</i> cells per patch				
1.245±0.071	1.367±0.100	1.196±0.051	1.298±0.091	1.217±0.063
Cells				
Observed mean patch size (total)	1.259±0.033			
Expected in random linear array	1.212			
Clone (observed/expected)	1.030±0.021			

Each of the five means is based on a complete sagittal section through the vermis of a chimera in which 17.5% of the cells were *Gus^b/Gus^b* (that is, stained). A total of 1,947 cells were scored. There were 270 patches (that is, groups of contiguous cells of like genotype). The mean patch size expected in a random linear array is estimated by $1/p$ where p is the proportion of cells of that genotype in the population ($p_{Gus^b} = 0.175$). For an approximation of the number of cells per patch in a two-dimensional array, the numbers could be squared. That would not, however, alter the conclusion that the distribution is near random.

cells of like genotype) with the mean expected in a random array⁹. In a purely random array, there would be one cell per clone. The data, shown in Table 1, yield an estimate of 1.03 Purkinje cells per clone. Thus, even in chimaeras in which there is no cell loss, the Purkinje cells seem to be almost randomly distributed. A fine-grained mosaicism has also been observed by Dewey *et al.*¹⁰ using other cell marking techniques.

These studies indicate that in the cerebellum the *pcd* locus exerts its primary effect within the Purkinje cell. The *pcd* locus is pleiotropic; its other effects include loss of retinal photoreceptor cells¹¹, mitral cells in the olfactory bulbs¹ and males have abnormal (or degenerating) sperm¹. These other effects were expressed in the chimaeras, but it could not be determined whether the *pcd* locus was also acting directly within those cell types.

It is well established that most cortical neurones, including Purkinje cells, are born in germinal centres such as the embryonic ventricular zone and then migrate to the position they will occupy in the adult cortex¹². Although they do migrate, the migration seems to be radial and one might expect that daughter cells would remain in proximity to one another which would give rise to clones of cells of the same genotype in adults. In this study, and in others^{10,13}, however, there is little evidence of clones which suggests that at some time in development there must be a considerable amount of cell mixing. Additional studies are obviously needed to confirm this finding and to determine when the mixing occurs. It will also be of interest to examine other cell types for Purkinje cells are unusual in that they are spread out into a single cell lamina during the massive growth and foliation of the cerebellum.

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Selective expression of xenotropic virus in congenic HRS/J (hairless) mice

GENETIC factors strongly influence susceptibility to leukaemia in mice. In some instances leukaemogenesis hinges on only a few genes, or even a single gene¹. The hairless mutation is an example of this. Homozygotes for this autosomal recessive gene (*hr/hr*) have a high incidence of thymic leukaemia (45% at 8–10 months), whereas the incidence in heterozygotes (*hr/+*) is low (1% at 8–10 months)². The leukaemogenic effect produced by homozygosity for *hr* is unknown. Here we report that thymuses of *hr/hr* mice produce high titres of xenotropic viruses, whereas thymuses of *hr/+* mice do not. Furthermore, viruses with a broadened host range were isolated from thymuses of preleukaemic *hr/hr* mice, but not from any tissue of *hr/+* mice. We propose that the mutant gene *hr* specifies circumstances that augment production of xenotropic virus by the thymus. This phenomenon may be an important step in the development of thymic leukaemia.

The virology of *hr/hr* and *hr/+* mice has not been extensively studied. Using a complement fixation test, Meier's group found that adult and embryo tissues of both homozygotes and heterozygotes contained similar amounts of murine leukaemia virus antigen³. But, using an XC plaque assay to test tail extracts the same authors later reported that the *hr/hr* mouse expressed several times higher amounts of ecotropic Type C virus than the *hr/+* animal³.

In a preliminary experiment we found that the ecotropic virus in HRS/J mice, the strain that carries the *hr* gene, was N-tropic. Therefore, we measured ecotropic virus in a modified XC plaque assay using NIH-3T3 cells⁴. As shown in Fig. 1a, N-tropic virus was readily detected in spleen, thymus, and bone marrow of both *hr/hr* and *hr/+* mice. Titres of virus increased with age, most notably in the thymus. But, at no time was there a difference in titre of infectious ecotropic virus between the two types of mice. It is therefore unlikely that the difference in the incidence of leukaemia can be explained by a difference in the expression of ecotropic virus.

Xenotropic virus was assayed by a focus-induction assay in Moloney sarcoma virus-transformed, non-producer cat cells⁵ and a fluorescent antibody focus method involving mink lung cells⁶. With the cat cell assay, we detected small amounts of xenotropic virus in thymuses of 2-month-old mice, but there was no difference between *hr/hr* and *hr/+* mice at this age (Fig. 1b). The titre of xenotropic virus in thymuses of 8-month-old *hr/hr* mice increased remarkably, however, whereas virtually no increase was found in *hr/+* mice. Low titres of xenotropic virus were also found in spleen and bone marrow of some *hr/hr* and *hr/+* mice. Therefore, increased expression of xenotropic virus was specifically related to the age, tissue and genotype of the mouse.

We confirmed this observation with the mink cell assay (Fig. 1c): at 8 months of age the expression of xenotropic virus in thymuses of *hr/hr* mice was, on the average, 100 times more than that in thymuses of *hr/+* mice. In other tissue, low titres of xenotropic virus were occasionally detected. We failed to find any clear-cut morphological changes in the infected mink cells.

We next characterised further the xenotropic virus that infected the mink cells. After several *in vitro* passages of the infected mink cells, the supernatant was filtered (Millipore, 45 µm) and used to infect fresh mink cells. After three or four more passages the culture supernatant was filtered and tested for infectivity on both mink and NIH-3T3 cells by means of the XC-plaque assay and the fluorescent antibody focus method. None of 48 isolates produced XC plaques on either NIH-3T3 or mink cells; 46/48 isolates

were pure xenotropic virus in that they failed to infect mouse cells, but readily infected mink cells (Table 1). But, 2/7 isolates from thymuses of 8-month-old *hr/hr* mice infected both mouse and mink cells. Although we have not yet cloned the latter isolates, it is unlikely that they are

simple mixtures of ecotropic and xenotropic viruses because: (1) they were infectious in mouse cells after having been passed more than 10 times in mink cells, and (2) they were totally negative in the XC test. We refer to these viruses as polytropic because of their broad host range and to

Fig. 1 Expression of endogenous viruses in HRS/J mice. Ecotropic and xenotropic viruses were quantitated by infectious centre assays; titres were expressed as $\log_{10}$ of the number of plaques or foci per 10^7 cells used in the assay. Black columns represent the virus titres of *hr/hr* mice and open columns those of *hr/+* mice. *a*, Ecotropic virus. In this assay⁴, 2×10^5 NIH-3T3 cells were plated in a 60-mm plastic Petri dish; 24 h later, the monolayer was treated with 2 ml of $25 \mu\text{g ml}^{-1}$ DEAE-dextran for 60 min at 37°C , rinsed, and infected with serial dilutions of washed lymphoid cells. After 5 d the infected indicator cells were exposed to ultraviolet light and overlaid with 1×10^6 XC cells. Two days later, the culture was methanol-fixed and Giemsa-stained and the number of plaques was counted. The medium was $4 \times$ Eagle's minimum essential medium with 10% unheated foetal calf serum. *b*, Xenotropic virus detected by the cat cell assay⁵. The indicator cat cell (8C) monolayer was prepared as above, treated with DEAE-dextran for 30 min, rinsed and infected. Transformed cell foci produced by rescued MSV pseudotypes were counted 12 days after infection. The medium was McCoy's 5A medium with 15% heat-inactivated foetal calf serum. *c*, Xenotropic virus by the mink cell assay⁶. Preparation of the indicator cell monolayer and method of infection was the same as in *b*. Cultures were made in duplicate; one with 20×20 mm coverslip and another without. On the fifth day, cells on the coverslip were acetone-fixed and stained with fluorescein isothiocyanate conjugated goat antiserum against Moloney virus (lot 5010101, NCI). Cultures without coverslips were maintained for 7–10 d to study possible morphological changes in infected mink cells and for subsequent isolation of the virus. The medium was McCoy's 5A medium with 10% unheated foetal calf serum. In all the infectious centre assays *in vitro*, medium was changed at 2–4 d intervals.

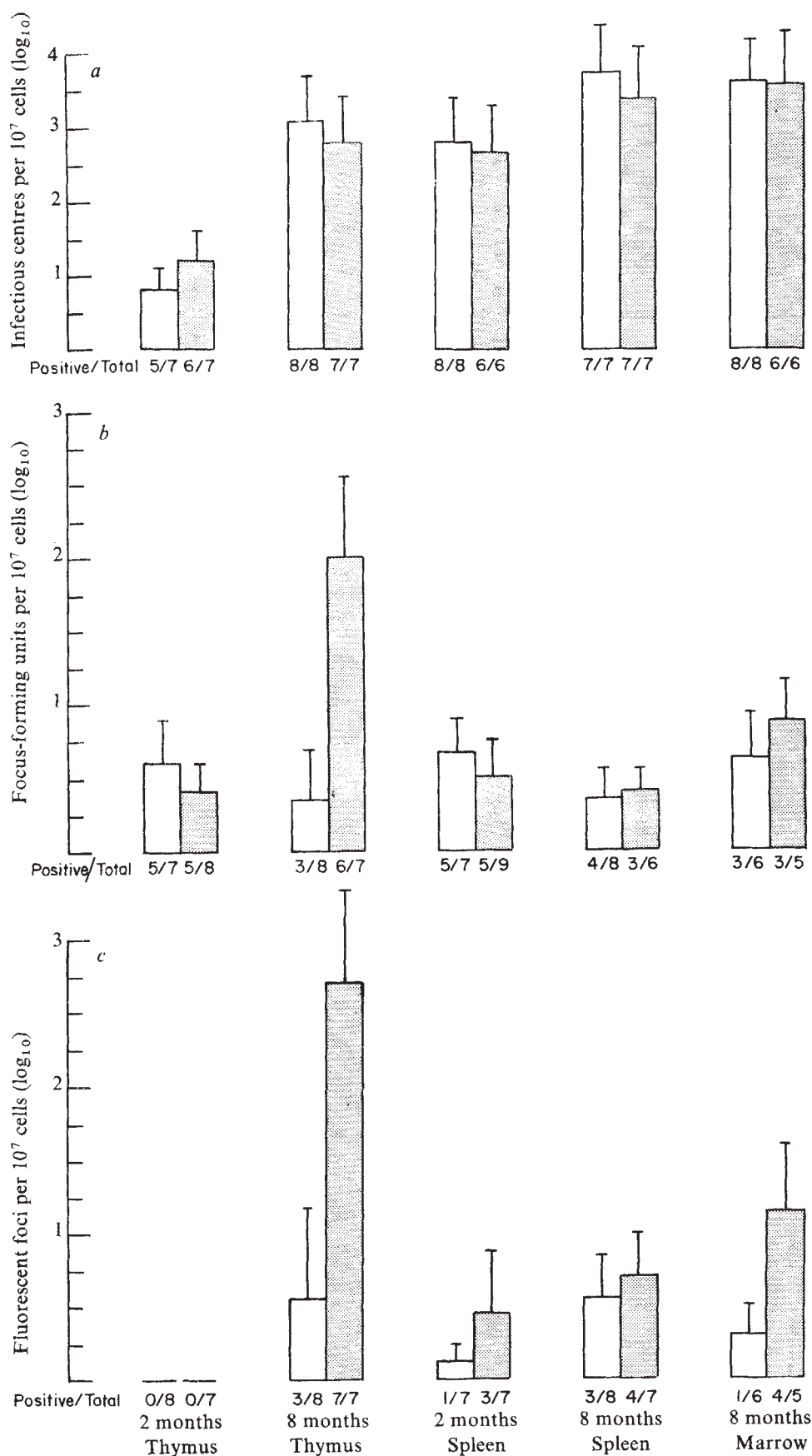


Table 1 Isolation of polytropic virus

Genotype	Age (months)	Polytropic/xenotropic		
		Thymus	Spleen	Bone marrow
<i>hr/hr</i>	2	0/2	0/3	0/3
	8	2/7	0/4	0/1
	Leukaemic	0/5	0/2	
<i>hr/+</i>	2	0/2	0/3	0/4
	8	0/3	0/1	0/3
	Leukaemic	0/2	0/3	

distinguish them from the amphotropic viruses of wild mice⁷. In a preliminary experiment, both an ecotropic and a xenotropic virus interfered with the infectivity of the polytropic virus for mouse and mink cells, respectively (data not shown). The polytropic isolates produced subtle morphological changes in infected mink cells, such as piling-up of cells or tiny cytoplasmic vacuolations, but these changes were not as clear as those reported by Hartley *et al.*⁸ with the analogous AKR virus. Results of further characterisation of the polytropic isolates will be reported elsewhere. Several xenotropic isolates obtained from leukaemic tissue were also studied; none of the 12 samples was polytropic.

Titres of ecotropic and xenotropic viruses in leukaemic tissues of *hr/hr* and *hr/+* mice are shown in Fig. 2. There were no significant differences in titres of ecotropic virus between preleukaemic and leukaemic thymic tissues. By contrast, leukaemic thymic tissue had much lower titres of xenotropic virus than preleukaemic thymus tissue. These findings may be explained either by regulation of expression of the virus at different stages of leukaemogenesis or by a shift in cell types in the thymus.

Our results are comparable to those of Hartley *et al.*⁸ in the AKR mouse. These workers demonstrated an increased

expression of xenotropic virus in thymuses of preleukaemic AKR mice⁶. Some isolates of this virus were polytropic and probably represent recombinant viruses⁸. The polytropic virus obtained from AKR mice produced no plaques in the XC test but productively infected mouse cells, as determined by an immunofluorescent method. The virus we obtained from hairless mice had similar properties. There are other similarities between the AKR and hairless systems: titres of ecotropic virus are high in all three types of mice; in contrast, expression of xenotropic and polytropic viruses is observed specifically in thymuses of AKR and *hr/hr* mice, but not in the *hr/+* mouse.

We stress here the outstanding advantage of the hairless mouse system: it is congenic, thus providing internal controls for analyses of the effects of one or at most very few genes in viral leukaemogenesis. Our results suggest that one effect of the mutant gene is to augment the production of xenotropic virus by the thymus. Genetic recombination between this agent and an ecotropic virus may give rise to the polytropic virus we detected. Experiments to test the oncogenicity of the latter agent are in progress.

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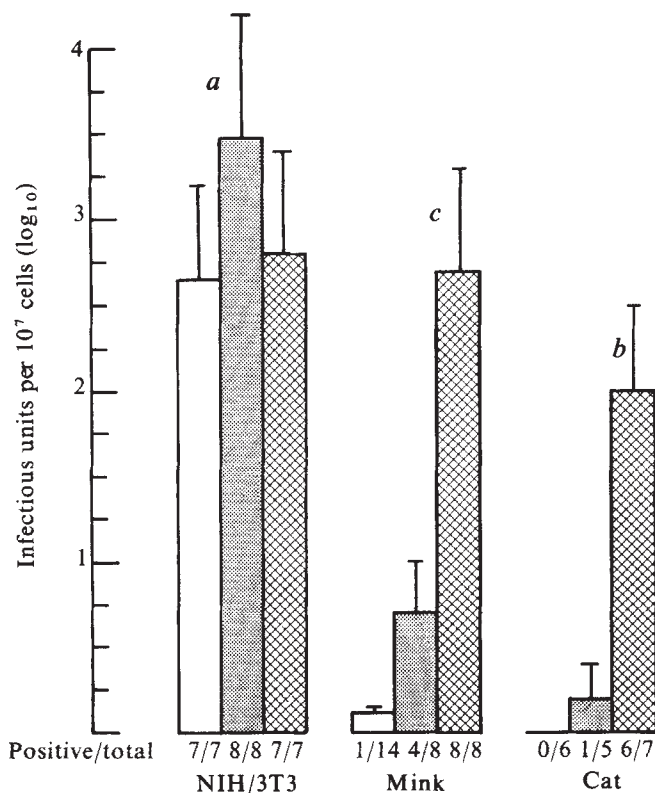
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Fig. 2 Ecotropic and xenotropic viruses in preleukaemic and leukaemic thymus. *a*, Ecotropic virus; *b*, xenotropic virus (cat cell assay), *c*, xenotropic virus (mink cell assay). Black column represents the virus titre in leukaemic tissue of *hr/hr*; open column, *hr/+* leukaemic tissue; and hatched column, the titre in preleukaemic *hr/hr* thymus.



A feline leukaemia virus- and sarcoma virus-induced tumour-specific antigen

BIOCHEMICAL and immunological methods of distinguishing between normal and malignant cells have had limited success. When studying cellular transformation, it is advantageous to study tumour viruses since they contain a definable quantity of genetic information for viral replication and for cell transformation. Viral gene products, such as viral structural antigens, can be studied in both normal infected and virus-transformed cells. Oncornavirus antigens are found in the cytoplasm and cell membrane of both normal and transformed infected cells^{1,2}. These antigens are glycoproteins of the viral envelope or are internal viral proteins³⁻⁵. Non-viral tumour-specific antigens resulting from transformation of the host cell by the causative virus, are found in the tumour cell membrane of some tumours induced experimentally by polyoma⁶ and SV40⁷ DNA viruses; and in cells transformed by an oncornavirus, the Rous sarcoma virus^{8,9}, but have not yet been found in any oncornavirus-induced leukaemic cell. Lymphosarcoma (LSA) or leukaemia of pet cats is caused by a contagiously spread oncornavirus, the feline leukaemia virus (FeLV)¹⁰. Antibody to the feline oncornavirus-associated cell membrane antigen (FOCMA)^{11,12} is associated with resistance to LSA development in FeLV-infected pet cats^{13,14} and with regression of feline sarcoma virus (FeSV) experimentally-induced fibrosarcomas¹⁵. To

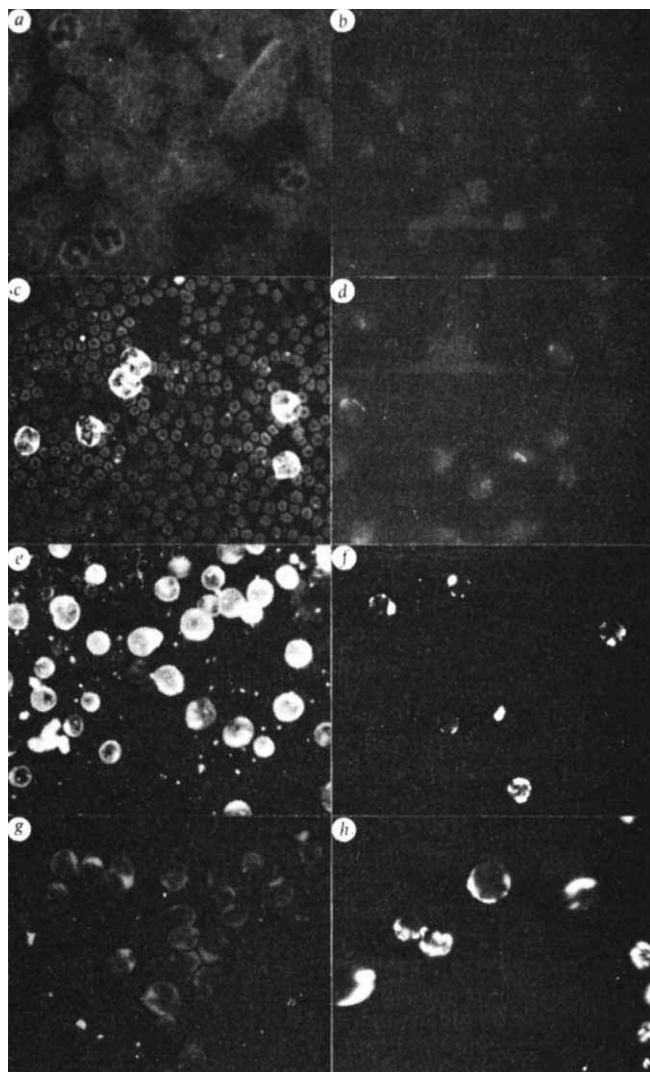


Fig. 1 Viable cell IFA tests for FOCMA and fixed cell IFA tests for intracytoplasmic FeLV antigens of cat cells. Normal feline leukocytes: *a*, FeLV antigen negative; *b*, FOCMA negative. Normal feline leukocytes: *c*, FeLV antigen positive; *d*, FOCMA negative. Lymphosarcoma cells: *e*, FeLV antigen positive; *f*, FOCMA positive. Lymphosarcoma cells: *g*, FeLV antigen negative; *h*, FOCMA positive. In the direct FOCMA test a 0.05ml aliquot of a 15×10^6 ml⁻¹ washed cell suspension was incubated at 4 °C for 30 min with 0.05 ml 1:4 dilution of FeLV-infected cat serum which had a high titre (1:32) of antibody to FOCMA, but which had no FeLV-neutralising antibody. As a control, another 0.05ml aliquot of a washed cell suspension was incubated, in the same conditions, with 0.05 ml cat serum containing no FOCMA antibodies. After incubation, the cells were washed twice, mixed with the fluorescein isothiocyanate-conjugated rabbit anti-cat serum globulin (Sylvania, Millburn, New Jersey), diluted 1:10, and allowed to react on ice for 30 min. Cells were then washed twice and resuspended in 0.05 ml 50% glycerin in PBS. A minimum of 100 cells were counted from each cell preparation. When 50% of the cells had punctate membrane fluorescence, and when the number of cells fluorescing in the FOCMA test was at least 25% greater than the number of fluorescing B cells from the same preparation, the cat was considered to have FOCMA-positive lymphoid cells (that is, the cells were lymphoid tumour cells). In the FOCMA absorption test, an aliquot of the cat anti-FOCMA serum was used at two double dilutions below the 50% fluorescent endpoint. The diluted serum was then absorbed twice for 30 min each at 4 and 37 °C with an equal volume (a minimum of 0.2 ml) of washed packed test cells. The residual FOCMA antibody titre of the absorbed cat anti-FOCMA serum was then determined in a direct FOCMA test as described previously, and compared with that of unabsorbed (control) serum from the same aliquot. A 25% or greater reduction in the FOCMA antibody titre of the absorbed serum was considered a positive absorption, indicating that FOCMA was present on the B lymphoid cells and that these cells were tumour cells.

determine whether FOCMA is a tumour-specific antigen, we tested normal, LSA and fibrosarcoma cat cells for both FeLV antigens and FOCMA. We report here that FOCMA is a tumour-specific antigen induced by naturally occurring feline leukaemia and sarcoma viruses. Our observation, which was made in a random bred species, the pet cat, may have particular relevance for the diagnosis and therapy of naturally occurring lymphoid tumours of other animals, including man.

Cells from both FeLV-infected and uninfected healthy pet cats, and FeLV-infected and uninfected cats with naturally occurring LSA, were studied. All cells were tested for cytoplasmic FeLV structural antigens by the fixed cell immunofluorescent antibody (IFA) test¹⁶. Detection of FeLV antigens in the cytoplasm indicates that FeLV is replicating in these cells and that FeLV gp70 and p30 are present in their cell membranes^{4,5,16}.

To determine whether FOCMA is expressed on lymphoid cells regardless of their cellular origin, tests to determine whether the cells were of T or B cell origin were also carried out. Lymphocytes were tested for their ability to form rosettes with guinea pig erythrocytes to determine whether they were of T cell origin, and for the presence of cell surface immunoglobulins (Ig) to determine whether they were of B cell origin¹⁷.

Two tests for FOCMA were carried out, the direct test and the absorption test using a standard reference serum (see Fig. 1)^{11,12}. This reference serum was obtained from an FeLV-infected cat and contained FOCMA antibody at a titre of 1:32 but did not contain FeLV-neutralising antibody. The FOCMA reactivity of the serum could not be absorbed out with intact or disrupted FeLV, with purified FeLV gp70 or p30^{18,19}, with foetal cat cells, or with normal cat lymphoid cells. FOCMA antibody was absorbed only by FeLV-producing or non-producing feline LSA cells. If the lymphoid cells were found to be of B cell origin they were tested for FOCMA by the absorption test. This was necessary since the fluorescein-conjugated rabbit anti-cat serum globulin used in the direct FOCMA test reacts with Ig-producing B cells and thus makes interpretation of the direct test impossible.

A total of 36 cats with histological diagnoses of LSA were studied. Twenty-seven of the cats had FeLV-producing LSA cells, whereas the LSA cells of the other nine cats were not replicating FeLV. Twenty-three out of the 36 LSAs (64%) were of T cell origin (Table 1). All 12 thymic LSAs and 10 of the 18 multicentric LSAs were T cell tumours. Both T and B cell markers were present in seven of the LSAs and were classified as mixed cell tumours. Only four LSAs were of B cell origin and three of these were LSAs of the gastrointestinal tract. No lymphocyte cell surface markers could be detected in two LSAs and these were classified as null cell tumours.

All 33 FeLV-producing LSA cell preparations obtained from 27 FeLV-infected LSA cats were positive for FOCMA (Table 2). Similarly, all 13 LSA cell preparations, which were not replicating FeLV, obtained from nine FeLV IFA test-negative LSA cats were also FOCMA positive. FOCMA was expressed on lymphoid tumour cells (Fig. 1) regardless

Table 1 T and B cell origin and forms of feline lymphosarcoma

Lymphosarcoma form	No. of cats tested	T	B	Mixed	Null
Multicentric	18	10	1	5	2
Thymic	12	12	0	0	0
Alimentary	5	1	3*	1†	0
Unclassified	1	0	0	1	0
Totals	36	23	4	7	2

* Two gastric LSA.

† Intestinal LSA.

Table 2 Occurrence of FOCMA in cats with lymphosarcoma

Number of cats with lympho-sarcoma	FeLV status of cats with lympho-sarcoma	Tissues tested	Number tested	FOCMA positive	FOCMA negative
27	+	Lymphosarcoma cells obtained from:			
		Blood	15	15	0
		Thymus	9	9	0
		Lymph Nodes	6	6	0
		Stomach	1	1	0
		Spleen	1	1	0
		Kidney	1	1	0
		Total	33	33	0
9	-	Normal blood lymphocytes from +cats with LSA	4	0	4
		Lymphosarcoma cells obtained from:			
		Lymph nodes	4	4	0
		Thymus	3	3	0
		Stomach or intestine	2	2	0
		Blood	2	2	0
		Omentum	1	1	0
		Spleen	1	1	0
		Total	13	13	0
		Normal blood lymphocytes from -cats with LSA	4	0	4

of their FeLV status and T, B, mixed or null cell origin. By contrast, four out of four FeLV-infected and four out of four FeLV-negative morphologically normal lymphocyte preparations, which were obtained from the peripheral blood of cats with LSA who had no evidence of circulating leukaemic cells, were negative for FOCMA. A total of 63 normal cell preparations, 20 FeLV-infected and 43 FeLV uninfected, obtained from healthy cats were negative for FOCMA. Normal lymphocytes from 11 FeLV-infected and 22 uninfected cats were FOCMA negative. Infected neutrophils from six healthy cats and infected thymocytes from three foetuses were FOCMA negative. Uninfected neutrophils (eight cats), thymocytes (five cats), splenocytes (five cats) and myelocytes (three cats) were also negative for FOCMA.

Normal FeLV-infected and uninfected feline embryo lung fibroblast cell cultures were negative for FOCMA. Similarly, three non-lymphoid feline tumour cell cultures (one mammary adenocarcinoma, one osteosarcoma, and one anaplastic sarcoma) were also negative for FOCMA. Two fibrosarcoma cultures obtained from young cats with naturally occurring multicentric FeSV fibrosarcomas and granulocytic tumour cells from a cat with myelogenous leukaemia were, however, FOCMA positive, as was a dog fibrosarcoma culture from an FeSV-induced fibrosarcoma in a beagle puppy.

Our findings indicate that FOCMA is an FeLV- or FeSV-induced tumour-specific antigen expressed on the membranes of naturally occurring feline LSA, myelogenous leukaemia and multicentric fibrosarcoma cells. The finding that myelogenous leukaemia cells are FOCMA positive is important, since it indicates that FeLV can transform granulocytes in addition to lymphocytes. Since FOCMA has been induced by FeSV in cells of other species²⁰, it is unlikely that FOCMA is an expression of an activated feline cellular gene. The expression of FOCMA on the membranes of FeLV non-producer feline LSA cells enables these cells to be used to study a defective leukaemia virus of a naturally occurring LSA. Such cells will be valuable

for comparative studies of lymphoid tumours of all animals, including man.

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Role of sendai virus fusion-glycoprotein in target cell susceptibility to cytotoxic T cells

IN mice, cytotoxic T lymphocytes (CTLs) are generated *in vivo* or *in vitro* against virally-infected syngeneic cells¹⁻⁴. The specificity of killing requires that the target cells possess both viral gene product(s) and H-2K and/or D gene products which are identical to those on the effector cells⁵. Target cells coated with non-infectious (ultraviolet-inactivated) Sendai virus are also specifically lysed by syngeneic virus-specific CTLs⁶. We show here that it is primarily the fusion glycoprotein of the Sendai virus envelope which is essential for the formation of the target antigen on cells coated with ultraviolet-inactivated Sendai virus.

The Nagoya strain of Sendai virus (from Dr H. Tozawa, Kitasato University, Japan), grown in 10-d-old embryonated chicken eggs, was purified and inactivated by exposure to ultra-

violet light. Ultraviolet-treated Sendai virus (SVuv) shows no infectivity (<5 PFU per ml, no plaques for a 0.2 ml undiluted sample) in primary rhesus monkey kidney cells and also no detectable synthesis of viral proteins in coated P815 (H-2^d) mouse mastocytoma cells (from Dr B. Smith, Institute for Cancer Research, Philadelphia) at 24 h after coating with virus as measured by direct FITC-antibody (Microbiological Associates), staining (data not shown). For the selective digestion of the fusion glycoprotein of the Sendai viral envelope, SVuv was treated with trypsin $20 \mu\text{g ml}^{-1}$ according to the method of Shimizu *et al.*⁷

Table 1 shows that trypsin treatment of SVuv resulted in an 88% decrease in cell-fusion activity and an approximately 95% decrease in haemolytic activity. Thus, trypsin inactivates the Sendai virus fusion and haemolytic activities, both of which reside on the viral fusion glycoprotein (referred to as the F glycoprotein)⁸. On the other hand, trypsin did not affect the viral haemagglutinin (HA) and neuraminidase (NA) activities which are both known to reside on the only other viral glycoprotein (referred to as the HANA glycoprotein)⁸ (Table 1). Results similar to these have also been observed by Shimizu *et al.*⁹. Thus, the two ultraviolet-treated Sendai virus preparations used for coating target cells differed in that one possessed the functional activities of the F glycoprotein (referred to as SVf⁺) and the other lacked the functional activities of the F glycoprotein following trypsin digestion (referred to as SVf⁻).

Sendai virus-specific cytotoxic effector cells were prepared using a modification of the method of Schrader *et al.*⁶. DBA/2J (H-2^d) mouse spleen cells, which had been primed *in vivo* with SVuv, were re-stimulated *in vitro* with γ -irradiated syngeneic spleen cells coated with SVuv. As a control, normal (unprimed) DBA/2J spleen cells were co-cultured with γ -irradiated normal C3H/HeJ (H-2^k) mouse spleen cells. Only SVf⁺ coated syngeneic P815 (H-2^d) cells were killed significantly by Sendai virus-specific sensitised DBA spleen cells. SVf⁻-coated and mock-infected P815 targets were not killed significantly by the effector cells (Fig. 1). Effector cells stimulated *in vitro* with normal allogeneic (C3H/HeJ) spleen cells did not significantly lyse the P815 target cells (Fig. 1). Therefore, the virus-specific lympholysis was not due to nonspecific killing caused by effector cells binding to target cells by Sendai virus bridges. After the *in vitro* generation, treatment of effector cells with anti-Thy-1.2 and complement showed that SVuv-specific killing was due to T cells (data not shown). These experiments were

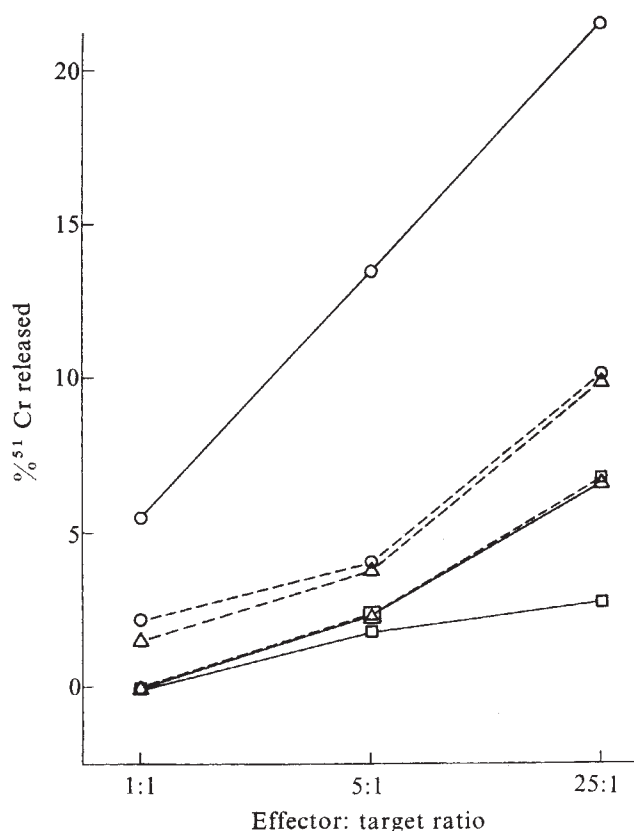


Fig. 1 Susceptibilities of P815 target cells coated with SVf⁺ or SVf⁻ to Sendai virus-specific CTLs. DBA/2J mice were primed *in vivo* with a single injection of 100–200 haemagglutinating units (HAU) of SVuv per mouse 3 weeks before collection of spleen cells. 5×10^7 spleen cells were cultured with 3×10^7 unprimed DBA/2J spleen cells pretreated with NH_4Cl to remove red blood cells, coated with SVuv at a concentration of 100–200 HAU per 1×10^7 cells (as described below), and γ -irradiated at 2,000 R. Using the procedure described above, unprimed DBA spleen cells were cultured with 5×10^7 normal C3H/HeJ spleen cells. The culture medium consisted of EHAA¹¹ supplemented with 5×10^{-3} M 2-mercaptoethanol and 5% heat-inactivated foetal calf serum. Effector cells were collected 6 d later from cultures containing the virus-coated syngeneic stimulating cells (—) and the allogeneic stimulating cells (---) by using Ficoll-Isopaque fractionation to remove red cells and dead cells¹². Target cells were prepared as follows: ^{51}Cr -labelled P815 cells were treated at 4°C for 1 h with SVf⁺ in PBS ($\circ$), SVf⁻ in PBS ($\triangle$), or PBS ($\square$) at 200 HAU per 1×10^7 cells. The cells were then washed twice with PBS. Cytotoxicity assays were performed in round bottom-wells of a microplate (Linbro) with 1×10^4 target cells and a given number of effector cells per well and were allowed to react for 6 h. The data are plotted as % ^{51}Cr -release for triplicate wells. The indicated s.d. was always less than 5%. The spontaneous release of all targets was 23–26%. Percentage cytotoxicity was calculated as follows:

$$\frac{\text{c.p.m. experimental release} - \text{c.p.m. spontaneous release} \times 100}{\text{c.p.m. maximum release} - \text{c.p.m. spontaneous release}}$$

Table 1 Effects of trypsin treatment on Sendai virus envelope activities

Sendai virus	HAU per ml	NAU per ml	% Cell fusion	% Haemolysis
Trypsin treated	1,900	58	12	4.8
Untreated	1,900	56	100	100

Sendai virus grown in embryonated chicken eggs was purified by discontinuous sucrose gradient (20% and 50% (w/w)) centrifugation and resuspended in phosphate-buffered saline (PBS) (pH 7.2). Aliquots of 2 ml of purified virus were irradiated in the ultraviolet at $800 \mu\text{W cm}^{-2}$ for 30 min with continuous rocking in a plastic dish (Falcon 3002). The ultraviolet-inactivated virus was treated with trypsin (DCC treated bovine pancreatic trypsin, Sigma) at a final concentration of $20 \mu\text{g ml}^{-1}$ at 36°C for 30 min. Trypsin was removed by centrifugation at $55,000g$ for 30 min at 4°C and the trypsin-treated (SVf⁻) and untreated virus (SVf⁺) were each resuspended in PBS. Haemagglutination (HA), neuraminidase (NA) and haemolytic assays were carried out as described previously¹⁰. The cell fusion activities of the virus preparations were expressed as the percentage decrease in the total number of cells in the virus-infected cultures (compared to mock-infected control cultures) due to the cell-to-cell fusions. For cell fusion measurements, replicate cultures of HeLa cells (seeded one day previously at 2×10^6 cells per 60 mm plastic Petri dish) were inoculated with 0.5 ml of SVf⁺, SVf⁻, or mock-infected. Following a 1 h virus adsorption, the cultures were overlaid with 5.0 ml of media containing 10% foetal calf serum. Total cell numbers were measured 2 h later.

repeated three times with identical results to those shown in Fig. 1. Thus, the Sendai virus F glycoprotein is the primary virion component necessary for the lysis of ultraviolet-inactivated virus-coated target cells by syngeneic CTLs.

Treatment of the Sendai virion with trypsin causes selective proteolytic cleavage(s) of the F glycoprotein⁷ and inactivates its biological activities of fusion and haemolysis (Table 1 and ref. 9) with no detectable effect on the amount of viral HANA glycoprotein on the virus-coated cells. Table 2 shows that there is no significant difference in the cell-associated NA activities between SVf⁺- and SVf⁻-coated cells for at least 6 h after virus coating, which corresponds exactly to the cytotoxicity reaction time (Fig. 1). Similar results to those in Table 2 were obtained in a repeat experiment. These experiments rule out the possi-

Table 2 Neuraminidase activities of Sendai virus-coated P815 target cells

Time after coating cells (h)	Neuraminidase activity (A_{540}) from cells coated with		
	SVf ⁺	SVf ⁻	No virus
0	0.462	0.491	0.112
3	0.229	0.326	0.112
6	0.223	0.275	0.111

P815 cells were treated with SVf⁺ in PBS, SVf⁻ in PBS, or PBS in the conditions described for the labelled target cell preparations (see Fig. 1 legend). After washing, the cells were incubated at 37 °C in the culture medium for the indicated times. Following incubation, 2.4×10^6 cells were pelleted and assayed for cell-associated viral neuraminidase activity using the assay method used for Sendai virions¹⁰.

bility that there is a difference in the amount of adsorbed viral HANA glycoprotein on the target cells coated with either SVf⁺ or SVf⁻, that is, that fusion activity determines the amount of virus adsorbed to the cell surface in these conditions.

During the course of these studies, Koszinowski *et al.*¹³ reported that target cells coated with a partially purified envelope fraction (containing both HANA and F glycoproteins from disrupted Sendai virions) were susceptible to syngeneic virus-specific CTLs. Thus, our studies confirm and extend these results by showing that it is the F glycoprotein of the Sendai virus envelope that has an essential role in the formation of the target antigen on the inactivated virus-coated target cells recognised by the syngeneic (ultraviolet-inactivated Sendai virus-specific) CTLs.

Two general hypotheses have been proposed to explain H-2 restriction in virus-specific lymphocyte cytotoxicity¹⁴. The CTLs could recognise the H-2K and/or D gene products which are modified by the virus (altered-self hypothesis) or the CTLs could recognise both H-2 and viral gene products (dual recognition hypothesis). Recently, Ennis *et al.*¹⁵ reported experiments which support the dual recognition of both H-2 and viral haemagglutinin antigens for influenza virus-specific CTLs. It is not known whether the H-2 and viral antigens were recognised by two separate receptors, one for the H-2 antigen and the other for the influenza virus haemagglutinin antigen, or whether there could be one receptor specific for some structural association between these two antigens¹⁴. A possible structural association of H-2 and murine leukaemia virus envelope glycoprotein on the surface of mouse tumour cells producing murine leukaemia virus has been suggested from co-capping and co-patching studies¹⁶.

There are several possible ways of explaining the role of the F glycoprotein in the Sendai virus-specific cytotoxic reaction. The fusion activity of the ultraviolet-inactivated Sendai virus may be required for an association of H-2 gene products and virion component(s). It is believed that Sendai virion components can disperse quickly throughout the cell membrane after fusion of the viral envelopes¹⁷. Following the dispersion of Sendai virion components, the F glycoprotein itself and/or other viral gene products could interact with H-2 gene products in the cell membrane. Alternatively, the fusion glycoprotein itself could directly become a part of the target antigen without the expression of its fusion activity.

Experiments are in progress to discriminate between these various possibilities by using purified subcomponents isolated from Sendai virions. We are also investigating the possibility that in conditions where cells are infected with live (not ultraviolet-inactivated) viruses other virion or viral-induced component(s) could also contribute to the formation of the target antigens recognised by syngeneic virus-immune CTLs.

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Isolation of separate Fc receptors for IgG complexed to antigen and native IgG from a murine leukaemia

RECENT studies have suggested that membrane receptors for the Fc region of IgG (Fc receptors) may be heterogeneous structures with distinctive binding properties¹⁻⁵. Walker has reported that a single cell line may possess separate Fc receptors for IgG molecules of different subclass¹, and Heusser *et al.* have demonstrated that macrophage-like cell lines may possess two distinct receptors: one which binds monomeric IgG2a, and another which binds to aggregates of all subclasses². Unless recently reported that a variant clone of the P388D1 mouse macrophage line had the same number of binding sites for IgG2a as the parent line, but only 10% of the binding sites for rabbit IgG in the form of soluble antigen-antibody complexes, suggesting the presence of two distinct Fc receptors³. The molecular identity of Fc receptors remains uncertain since few structural studies defining these membrane components are currently available⁶⁻⁸. In this study we demonstrate that several glycoproteins with Fc binding activity can be isolated from the plasma membranes of L1210 cells, and provide structural evidence that separate receptors may be responsible for binding IgG which has been complexed with antigen, and monomeric or aggregated IgG.

The murine leukaemia L1210 was grown in stationary culture in RPMI 1640 supplemented with 10% foetal calf serum (Flow Laboratories, Rockville, Maryland). Cells (2×10^{10}) were swelled in hypotonic buffer and ruptured in a Ten Broeck homogeniser. The plasma membranes were prepared by a partition of the cell lysates in a two-phase system of polyethylene glycol and dextran⁹. The glycoproteins were extracted from the plasma membranes using 0.3 M lithium diiodosalicylate as described by Marchesi and Andrews¹⁰. In some experiments, the cells were labelled with ¹²⁵I using lactoperoxidase-catalysed iodination before lysing, and in other experiments the glycoproteins were labelled with ¹²⁵I by the chloramine-T method¹¹ after the extraction. In the two experiments in which lactoperoxidase-catalysed iodination was used, 39% and 46% of the cell-associated counts were recovered in the plasma membrane interphase, and 43% of the plasma membrane-

associated counts were recovered in the glycoprotein extract. The results obtained using the two labelling techniques were identical.

The glycoprotein extract obtained from 2×10^{10} cells contained approximately 2 mg protein and seemed to have Fc receptor activity since it was bound by erythrocytes coated with IgG antibody, but not by erythrocytes alone. The addition of 1 μ g of the glycoprotein extract to 10^6 L1210 cells and 10^8 IgG-sensitized erythrocytes resulted in 73% inhibition of EA rosette formation.

Purified human IgG (Miles Laboratories, Elkhart, Indiana) was extensively absorbed with viable L1210 cells and an $F(ab')_2$ fraction prepared by pepsin digestion and Sephadex G-200 fractionation¹². Purity of the intact and digested IgG was determined using SDS-polyacrylamide gel electrophoresis¹³. Cyanogen bromide-activated Sepharose 4B was coupled to either heat-aggregated human IgG, heat-aggregated $F(ab')_2$ human IgG, or keyhole limpet haemocyanin (KLH) as described previously⁶, and five columns containing 2 mg of coupled protein were prepared. Rabbit anti-human IgG (Cappel Laboratories, Downingtown, Pennsylvania) was added to one of the columns containing coupled aggregated human IgG, and rabbit anti-KLH was added to one of the KLH-coupled columns, so that two columns contained antibody complexed to antigen. The other columns contained only coupled KLH, aggregated human IgG or aggregated $F(ab')_2$ IgG. All columns were washed with phosphate-buffered saline (PBS) until the A_{280} in the eluate was equal to a blank control.

Equal amounts of radioactive glycoprotein extract in PBS were applied to each column and after a 60-min incubation at 23 °C the columns were washed with PBS until the c.p.m. in the eluates were equal to background. The columns and washes were counted and the percentage c.p.m. bound was calculated (Table 1). Those columns containing Sepharose 4B coupled to IgG with an intact Fc region bound between 35–44% of the labelled preparation, whereas the $F(ab')_2$ IgG and KLH columns bound only 2.8% and 4.4%, respectively. Elution of the columns with 0.1 M citrate buffer (pH 3.2) containing 0.5 M NaCl removed most of the radioactive material which was bound to the columns that contained an intact Fc region (Table 1).

The citrate eluates were analysed using SDS-polyacrylamide gel electrophoresis (Fig. 1). No distinct components were detected in the citrate eluates from the $F(ab')_2$ IgG-coupled or the KLH-coupled columns. Three distinct peaks with apparent molecular weights of 65,000, 45,000 and 28,000 (designated FI, FII and FIII) were found in the eluate from the aggregated IgG-coupled column (Fig. 1). An identical pattern was observed when non-aggregated IgG was coupled to Sepharose 4B. The citrate eluates from the columns containing immune complexes (IgG-anti-IgG or KLH-anti-KLH) revealed the same three peaks and an additional peak (designated peak A) with an apparent molecular weight of 125,000 (Fig. 1). The possibility that peak A represents a gel artefact due to overloading with IgG eluted from the complex columns was discounted by the fact that only a faint Coomassie blue-positive IgG band was present on the gels. Furthermore, gels loaded with one tenth the radioactive material revealed no IgG staining band, but the continued presence of peak A.

Peak A, FI, FII and FIII were purified by electroelution from polyacrylamide gels¹⁴ and each fraction contained less than 8%

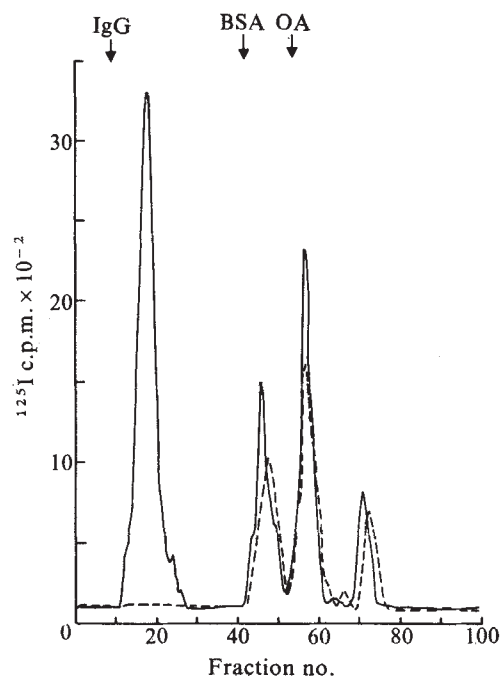


Fig. 1 Analyses of the SDS-polyacrylamide gel electrophoreses of the citrate eluates derived from Sepharose 4B columns coupled to aggregated human IgG to which anti-human IgG had been added (—), or to aggregated human IgG alone (---). The radioactive samples, in non-reducing conditions, were applied to parallel 7.5% acrylamide gels. Electrophoreses were carried out in non-reducing conditions to maintain the Fc binding properties of the proteins which were subsequently purified from the gels by electroelution. The citrate eluate from the KLH-coupled column, to which anti-KLH had been added, revealed an identical profile to the aggregated IgG-anti-IgG column. The citrate eluate from a non-aggregated IgG-coupled column had the same pattern as the aggregated IgG-coupled column. Peak A, which was demonstrated only in the citrate eluate of columns containing immune complexes, migrates in front of IgG and has an apparent molecular weight of 125,000. FI, FII, and FIII, with apparent molecular weights of 65,000, 45,000 and 28,000, were found in the citrate eluates from all columns that contained an intact Fc region. The citrate eluates from the $F(ab')_2$ IgG-coupled and KLH-coupled columns revealed no distinct peaks.

cross contamination from the other fractions. FI, FII and FIII revealed no change in molecular weight after reduction and alkylation. Reduction and alkylation of peak A resulted in the appearance of two radioactive peaks with apparent molecular weights of 74,000 and 60,000.

The ability of non-aggregated mouse myeloma proteins of different IgG subclasses to bind to intact L1210 cells was determined by indirect immunofluorescence. As judged by the intensity of the fluorescence, Table 2 shows that L1210 binds IgG preparations in the following order: human IgG = mouse IgG2a > mouse IgG1 > mouse IgG2b. Affinity columns of Sepharose 4B coupled to these same proteins were prepared and the percentage labelled peak A, FI, FII, and FIII bound by these columns determined (Table 2). All isolated glycoproteins bound better to human IgG or mouse IgG2a than to mouse IgG1 or IgG2b.

Table 1 Binding of 125 I-labelled glycoprotein extract to Sepharose 4B columns coupled to different IgG preparations

Buffer	% c.p.m. bound				
	Aggregated $F(ab')_2$ IgG	Aggregated IgG	Aggregated IgG + anti-IgG	KLH	KLH + anti-KLH
PBS, 0.01 M (pH 7.2)	2.8	35.7	44.1	4.4	42.7
Citrate, 0.1 M NaCl, 0.5 M (pH 3.2)	1.6	8.4	6.7	4.2	10.1

Equal amounts of radioactive glycoprotein extract in PBS were applied to the columns. After extensive washing, so that the counts in the eluate equalled background, the columns and washes were counted and the percentage of the applied c.p.m. bound was calculated. The columns were then eluted with citrate buffer and the percentage of applied c.p.m. remaining on the columns was calculated.

Table 2 Correlation of IgG subclass binding of intact L1210 cells and isolated Fc binding proteins

Sample	Human IgG	Mouse myeloma proteins		
		IgG1	IgG2a	IgG2b
L1210 Cells	++++	++	++++	+
Peak A	74.8	32.8	61.2	26.2
FI	59.6	40.8	63.8	32.6
FII	66.6	26.9	64.9	35.6
FIII	45.5	30.4	46.6	30.0

L1210 cells were incubated with either human IgG or one of the mouse myeloma proteins, washed, and then stained with the appropriate fluorescein conjugate (anti-human IgG or anti-mouse Ig). The intensity of the fluorescence was graded from + (barely visible) to ++++ (most intense). Peak A, FI, FII and FIII were passed over Sepharose 4B columns to which equal amounts of human IgG or one of the myeloma proteins had been coupled. The columns were washed with PBS until the c.p.m. in the eluates were equal to background. The numbers refer to the percentage of counts bound by each column.

These experiments indicate that four proteins which bind to the Fc region of IgG can be isolated from the glycoprotein extract of the plasma membranes of L1210. These proteins exhibit the same IgG subclass affinity as the intact cell, indicating their relationship to the cell's Fc receptors; however, their relationship to one another has not been determined. FI, FII, and FIII are composed of single polypeptide chains of similar amino acid composition, yet they differ considerably in their apparent molecular weights. Preliminary data suggest that the apparent differences may be due to either differences in the carbohydrate moieties of the molecules, or aggregation which is induced during the isolation procedure on SDS gels¹⁵. The smaller proteins may also represent degradation products of a larger Fc receptor. We consider this possibility less likely, since the addition of proteolytic inhibitors throughout the isolation procedure does not alter the results, and the isolated molecules still retain their Fc-binding properties.

Frøland *et al.*⁴ have presented data which suggest that the cells that form EA rosettes and those that bind aggregated IgG may be separate. Our data may provide a molecular basis for explaining these observations. The Fc binding proteins (FI, FII and FIII) isolated from L1210 bind to monomer or heat-aggregated IgG, whereas peak A seems to have specificity for IgG complexed to antigen. Thus, the ability to form rosettes with IgG-coated erythrocytes could depend on the possession of an Fc receptor which binds IgG antibody complexed to antigen, and this receptor may be distinct from the Fc receptor which binds IgG that is not complexed to antigen. It is also possible that the smaller Fc binding molecules which we have isolated may be related to each other and may be derived from a larger, complex Fc receptor. The different binding specificities exhibited by an individual cell could then be accounted for by the way in which these molecules are arranged or linked to one another on the cell's surface.

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Antagonism of antigen-induced contraction of guinea pig and human airways

EXPOSURE of isolated sensitised guinea pig or human airway tissue to specific antigen produces a rapid and prolonged contraction which has been studied as a model of asthmatic bronchospasm. This response is thought to be mediated by endogenous chemicals released from airway mast cells, since antigen treatment of sensitised guinea pig or human pulmonary tissue results in the elaboration of several substances, including histamine and slow reacting substance of anaphylaxis (SRS-A), which can contract airway smooth muscle. The few attempts at investigation of which mediators are responsible for the airways response have led to contradictory conclusions. Schild *et al.*¹ reported that antihistamines significantly (but not completely) antagonised antigen-induced contraction of bronchi from an asthmatic patient. Sheard and Blair², however, demonstrated that antihistamines had no effect on the contraction of human bronchial strips studied in the presence of antigen-challenged, passively-sensitised human lung. They suggested, without direct evidence, that the contractile response of the bronchial strip was the result of SRS-A release. At present, therefore, the roles of histamine and SRS-A in the airway response to antigen have not been defined. We have investigated the effects of diphenhydramine, a histamine H₁ antagonist, and FPL 55712 (ref. 3), an SRS-A antagonist, on the response of sensitised guinea pig and human airways to antigen and report that pretreatment with either alone modifies the airway response in a characteristic fashion, and that together, they effectively inhibit antigen-induced airway constriction.

Tracheas of male Hartley guinea pigs passively sensitised with rabbit anti-dog albumin were cut into rings which were tied together to form a chain and placed in an organ bath containing Tyrode's solution. Three chains of three rings each were constructed from each trachea and were studied simultaneously. Human bronchial tissue obtained from patients undergoing surgical procedures was sensitised by incubating with serum from a ragweed allergic subject. Circumferential bronchial strips, studied in duplicate or triplicate, were suspended in an organ bath containing Krebs-Henseleit solution. Isometric airway tissue contractions were recorded.

Exposure of either tissue to specific antigen resulted in a rapid contraction equivalent in amplitude to approximately 40-100% of maximum contraction. Unsensitised tissue did not respond. Although the amplitude of the antigen-induced contraction varied considerably from day to day, within each experiment the responses of the chains or strips from one smooth muscle preparation were approximately equal. The duration of the response, ranging from 30 to 150 min, was proportional to the amplitude and was essentially unaffected by repeated washings.

Pretreatment of guinea pig or human airway tissue with diphenhydramine delayed the onset of the response to antigen and decreased the rate of development of the contraction (Fig. 1A). Within each experiment, the effects of diphenhydramine were dose dependent, with effects noted at concentrations as low as 10⁻⁷ M. Pretreatment with doses of diphenhydramine as high as 10⁻⁴ M, however, did not significantly decrease the amplitude or duration of the antigen-induced contraction. Higher concentrations caused no specific effects, but even 10⁻³ M diphenhydramine had no effect when added after the airway response to antigen had developed.

Pretreatment with FPL 55712 in doses up to 5 × 10⁶ g ml⁻¹ did not significantly alter the initial phase of the airways

response to antigen but produced a dose-related decrease in amplitude and duration of the prolonged phase of the response (Fig. 1*B,b*). Higher concentrations of FPL 55712, in addition to further depressing the amplitude and duration of the prolonged phase, inhibited to some extent the initial phase of the antigen-induced contraction

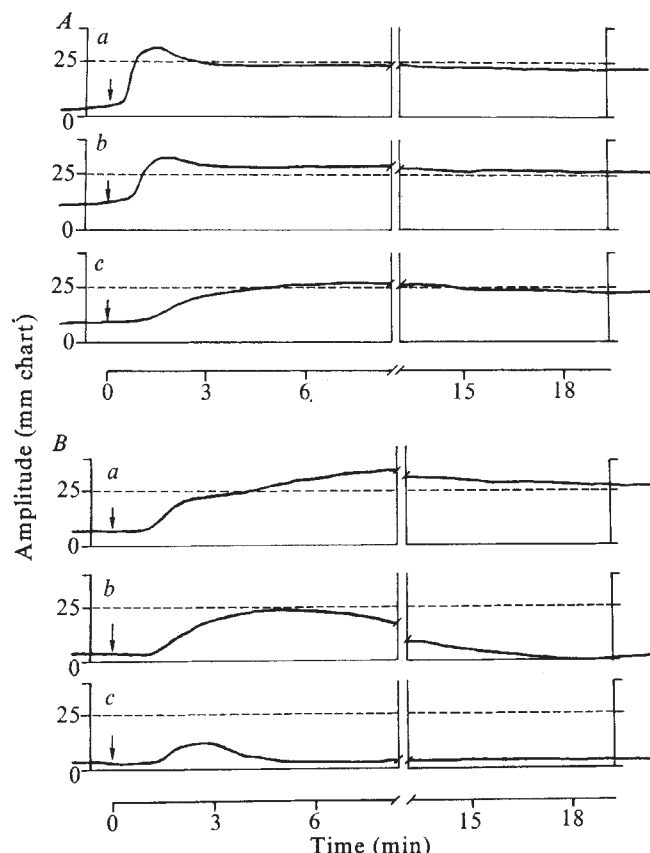


Fig. 1 Effect of, *A*, diphenhydramine, and *B*, FPL 55712, on the response of sensitized guinea pig trachea to antigen. *A*, Male Hartley guinea pigs (300–350 g) were passively sensitized by injecting 0.2 ml rabbit anti-dog albumin (Cappel Laboratories, Cochranville, Pennsylvania, 2 mg antibody protein ml⁻¹) intraperitoneally (i.p.) 12–16 h before use. After the animals were killed (pentobarbital, 90 mg kg⁻¹ i.p.), the tracheas were removed and cut into rings 2 mm wide. These were tied together to form a chain and placed in a 37°C organ bath containing Tyrode's solution bubbled with 95% O₂–5% CO₂. Three chains of three rings each were constructed from each trachea and were studied simultaneously. Airway tissue contractions were measured with Grass FT03C force-displacement transducers and recorded on a Grass Model 7 polygraph. After the initial tension was adjusted (1 g), the tissue was washed and treated with several doses of methacholine (2.5 × 10⁻⁶ M), until a consistent response was established. This dose of methacholine produced a contraction of the guinea pig airway tissue approximately equivalent to 75% of maximum, defined in our experiments as the response to 10⁻³ M methacholine. Amplifier gain was adjusted to give equivalent recorded contraction amplitude for each chain to the test dose of methacholine. The tissue was then washed and incubated for 10 min with diphenhydramine (*b*, 10⁻⁷ M; *c*, 10⁻⁵ M). *a*, Untreated control tissue. Dog albumin (10⁻⁶ g ml⁻¹) was added to each bath at the arrow. Control response to antigen was equivalent to 56% maximum contraction. Diphenhydramine produced a delay in onset of response to antigen (100% greater than control for 10⁻⁷ M, 200% for 10⁻⁵ M) and a decrease in initial rate of contraction. Duration of response (120 min) was unaffected. *B*, Tissue preparation as *A*, except that tracheal rings were incubated with FPL 55712 for 10 min (*b*, 2 × 10⁻⁶ g ml⁻¹; *c*, 2 × 10⁻⁵ g ml⁻¹). *a*, Control response of untreated tissue. Dog albumin (10⁻⁶ g ml⁻¹) was added to each of the baths at the arrow. Control response to antigen was equivalent to 60% maximum contraction. In contrast to antihistamine (*A*), FPL 55712 decreased duration of response to antigen by 85% (2 × 10⁻⁶ g ml⁻¹) and 95% (2 × 10⁻⁵ g ml⁻¹). Control duration was 110 min. Decrease in amplitude of early phase of response (*c*) was a result of antihistamine-like properties of FPL 55712.

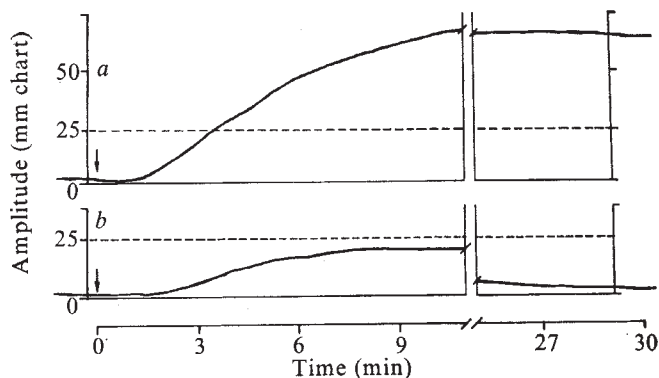


Fig. 2 Effect of diphenhydramine in combination with FPL 55712 on sensitized human airways response to antigen. Human bronchial tissue, obtained from a patient undergoing surgery, was passively sensitized by incubating overnight at 25°C with serum from a ragweed allergic subject (1:40 dilution in a balanced salt solution). Serial circumferential subsegmental bronchial strips were suspended in organ baths containing Krebs–Henseleit solution, bubbled with 95% O₂–5% CO₂ and maintained at 37°C. Tissue contractions were measured as described in Fig. 1. After a consistent response to methacholine (2.5 × 10⁻⁶ M) was established and amplifier gain was adjusted to give equivalent recorded contractions to the test dose of methacholine, the tissue was washed and the bronchial strip depicted in *b* was incubated with diphenhydramine (10⁻⁵ M) combined with FPL 55712 (10⁻⁵ g ml⁻¹) for 10 min. *a*, Control response of untreated tissue. Antigen E (5 × 10⁻⁷ g ml⁻¹) was added to each of the baths at the arrow. Control response to antigen was equivalent to 84% maximum contraction. Combination of antagonists produced delay in onset of response, decrease in amplitude of early and prolonged phases of contraction and a decrease in duration. Control response duration was 150 min.

(Fig. 1*B,c*). This effect is attributable to the reported^{3,4} antihistamine activity of FPL 55712. FPL 55712 added during the prolonged phase of antigen-induced contraction of guinea pig or human airway tissue reversed the response, the dose required being somewhat higher than that effective in pretreatment.

Diphenhydramine (10⁻⁵ M) in combination with FPL 55712 (10⁻⁵ g ml⁻¹) administered before the antigen regularly reduced both the amplitude and duration of the antigen-induced contraction to less than 30% of control, with complete blockade frequently being observed. Figure 2 shows the effect of pretreatment with both diphenhydramine and FPL 55712 on the response of human bronchial tissue to antigen. The extent of inhibition by this combination of antagonists depended on the relative amplitude of the contraction.

These data represent the first clear evidence that both histamine and SRS-A contribute to antigen-induced contraction of sensitized airway tissue and have important implications for the pharmacological control of human asthma. Histamine is primarily involved in the early phase of the response, commencing 1–2 min following the administration of antigen. This is the period most frequently examined in screening drugs by antigen bronchoprovocation in man. The effect of histamine was particularly obvious in the pattern of contraction of guinea pig trachea, where the response frequently showed two amplitude maxima, one occurring within 3 min of antigen administration and the other occurring after about 10 min. Pretreatment with diphenhydramine selectively abolished the early contraction maximum without altering the amplitude or duration of the later phase of the response. Maximal inhibition of the early phase of the response required about 10⁻⁵ M diphenhydramine. From data on the antagonism of exogenous histamine it seems that the mean effective tissue concentration of histamine in the region of the smooth muscle cells must be quite high, probably approaching 10⁻³ M.

SRS-A seems to contribute to the later, prolonged phase of the airway response to the antigen, since this phase

is effectively antagonised by FPL 55712 but not by diphenhydramine. The dosages of FPL 55712 required to produce a maximal effect were considerably higher than those reported by Augstein³ or Orange⁴ to inhibit SRS-A-induced contraction of guinea pig ileum. This is not surprising since, like that of histamine, the tissue concentration of SRS-A following antigen treatment must be many orders of magnitude higher than that achieved by the addition of exogenous SRS-A to an organ bath. Whether other mediators contribute to the response or modulate the airways reactions to histamine or SRS-A is not yet clear, but it seems evident that pharmacological control of human airways reactions *in vivo* will require either a drug which inhibits the release of multiple mediators or a combination of specific antagonists.

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An octopaminergic neurone modulates neuromuscular transmission in the locust

OCTOPAMINE, a biogenic amine, occurs widely in the nervous systems of vertebrates and invertebrates¹⁻³. It has been found in single neurones in the central nervous system of the marine mollusc, *Aplysia*⁴ and also in peripheral neurosecretory neurones in the lobster^{5,6} from which a calcium-dependent release of octopamine has been demonstrated^{7,8}. Specific octopamine receptors⁹ and octopamine activated adenylate cyclases¹⁰ have also been found in invertebrate nervous tissue. This has led to the suggestion of a neurotransmitter role for octopamine in invertebrates¹⁻³. In the absence, however, of an example in which the effect of stimulating an identified octopamine containing neurone on its identified target or end organ is known, the true function of octopamine remains obscure. This paper aims to demonstrate that octopamine is the neurotransmitter of a single identified neurone in the locust *Schistocerca americana gregaria*. This neurone, which is shown to contain octopamine, innervates a muscle where it functions as a neuromodulator by potentiating the synaptic potential and tension produced by an identified motoneurone. That this function is mediated by the release of octopamine on stimulation is supported by the action of exogenously applied octopamine.

The extensor-tibiae muscle of the locust hind leg is a large and powerful muscle used in walking, kicking and jumping. It is innervated by three motoneurones¹¹⁻¹³ whose axons are contained in the extensor-tibiae nerve. They are the fast and the slow extensor motoneurones (FETi and SETi) and an inhibitory motoneurone which innervates many muscles and has, therefore, been called the common

inhibitor (CI). These neurones are paired and so there are six identified motoneurones controlling the paired (left and right) extensor muscles. Their cell bodies lie in known positions in the metathoracic ganglion (Fig. 1a). The muscle also receives a fourth axon¹⁴ belonging to a single unpaired

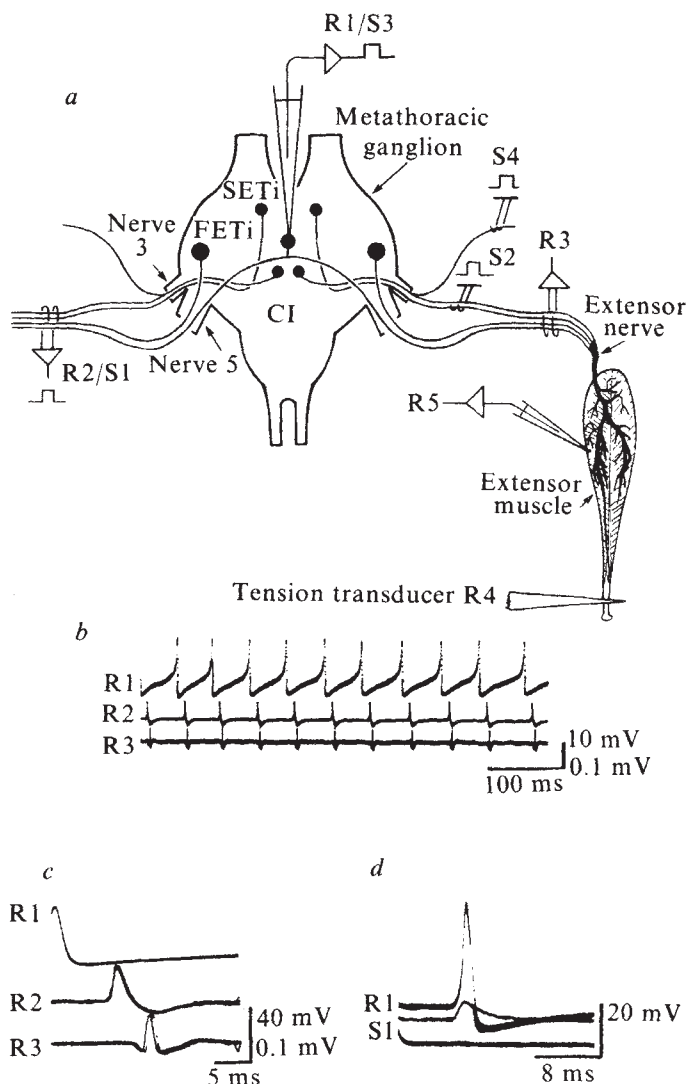


Fig. 1 The experimental arrangement and the identification of DUMETi. *a*, Recording (R) and stimulating (S) sites referred to here and in Fig. 3. ●, Positions of the neurone cell bodies in the locust metathoracic ganglion which have axons in the extensor tibiae nerve. Independent stimulation of each axon in the extensor nerve was achieved as follows. The DUM axon was stimulated either by passing current into the soma of DUMETi (S3) or by antidromic stimulation of the left extensor nerve (S1). Selective stimulation of the axon of the slow extensor motoneurone (SETi) in a branch of nerve 3 (S2) which contains both its axon and that of the common inhibitory motoneurone (CI) is achieved by the relatively larger diameter of the former which gives it a lower threshold to extracellular stimulation. The common inhibitory motoneurone was stimulated in a branch of nerve 3 (S4) which contains its axon but lacks other axons which project to the extensor muscle. Muscle tension is monitored almost isometrically with a tension transducer placed on the muscle tendon (R4). *b*, Spikes initiated in the soma of DUMETi (R1/S3) are transmitted 1:1 to both left and right extensor nerves. This distinguishes the DUMETi neurone soma from those of other DUM cells. *c*, The oscilloscope is triggered several times by spikes recorded in the soma of DUMETi, note that spikes on the left (R2) and right (R3) axons superimpose. The difference in absolute latency between spikes recorded left and right is caused by a difference in the distance from the soma of sites R2 and R3. *d*, The soma spike can be initiated antidromically by stimulating either left or right extensor nerves. If the soma is hyperpolarised, the active soma spike fails to initiate in response to antidromic stimulation (R1, lower trace). The smaller positive potential seen in the soma is a recording of the axon spike conducted passively from its site of initiation.

neurone with its cell body among a group of similar unpaired neurones in the dorso-medial region of the metathoracic ganglion^{15,16}. They are called Dorsal Unpaired Median or DUM neurones and the one which innervates the extensor-tibiae muscle is known as DUMETi¹⁴.

The DUM cells stain selectively with the dye neutral red (Fig. 2), a dye known to stain monoamine-containing neurones in the leech¹⁷ and lobster nervous systems^{5,6}. This finding, together with the observation that tissue removed from the dorsal part of the locust metathoracic ganglion, possibly containing DUMETi, is capable of octopamine synthesis from tyrosine¹⁸, directed our attention towards the identification of a biogenic amine, possibly octopamine, as a neurotransmitter for DUMETi. We identified the DUMETi neurone by intracellular recording (Fig. 1 *b-e*) in 50 animals. The soma was removed, cleaned of adhering pigment cells and other tissue and transferred to a microtube containing 10 μ l of 0.01M formic acid. Five assays were performed on pools each containing 10 identified DUMETi cell bodies. Octopamine was assayed by a modification (P.D.E. in preparation) of the standard radio-enzymatic assay¹⁹ which detected as little as 10 pg of DL-octopamine. The identity of the assay products was checked by several chromatographic and electrophoretic techniques⁶. The mean amount of octopamine found per DUMETi soma ($\pm$ s.e.m.) was 0.10 ± 0.02 pmol. Control assays performed on pools of up to 37 fast extensor motoneurone cell bodies gave no detectable octopamine. The minimum concentration ratio between the two neuronal types is therefore 800:1. Octopamine was also detected in pieces of extensor nerve which contained the axon of DUMETi but was undetectable in pieces of the same nerve which on physiological evidence were known to contain only the axons of the slow extensor and common inhibitor motoneurones. Of the neurones innervating the extensor muscle, therefore, only DUMETi contains octopamine. Octopamine is about four times more concentrated in the axon of DUMETi than in its soma.

We were first alerted to the possibility that DUMETi is

Fig. 2 A dorsal view of a locust metathoracic ganglion showing the medial group of cell bodies stained with the dye neutral red (0.01 mg ml^{-1} in isotonic locust saline for 3 h at room temperature). The group contains two quite distinct size classes, large (L) and small (S) cells. The soma of the DUMETi neurone is a member of the group of large cells. The organisation of the group is variable, the soma of DUMETi can be found anywhere among the group of large cells. Compare ganglion outline with Fig. 1a.

A, Anterior; P, posterior. Scale bar, 100 μ m.

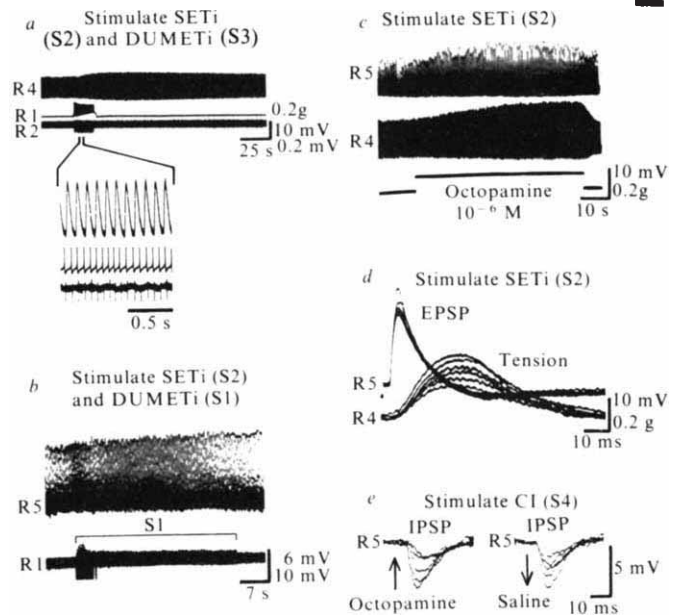
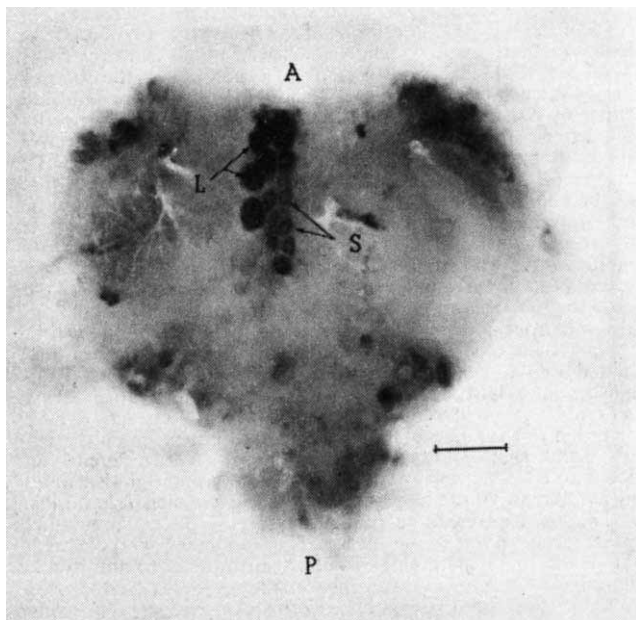


Fig. 3 *a*, The effect of stimulating DUMETi on extensor muscle tension generated by the slow extensor motoneurone. The time base is expanded below to show the individual twitches of the muscle (R4), the soma spikes (R1) and axon spikes (R2) of DUMETi. The slow motoneurone was stimulated at 10 Hz. Note the slow potentiation and long lasting effect of DUMETi on tension. *b*, The effect of stimulating DUMETi on EPSP amplitude (R5). DUMETi is stimulated antidromically (S1) at 20 Hz. At this frequency the soma spikes are unable to follow 1:1 and fail to initiate at all after the first few seconds of stimulation. A few soma spikes can be seen during the initial seconds of stimulation (R1); subsequently only the smaller axon spike is recorded in the soma. *c*, The effect of 10^{-6} M DL-octopamine in saline on the EPSP (R5) and tension (R4) generated by stimulating the slow motoneurone (S2). *d*, The oscilloscope is triggered by the stimulus delivered to the slow motoneurone (S2). Each EPSP (R5) produces a response in the tension transducer (R4). A series of six triggered sweeps about 5 s apart starting at the arrival of octopamine are shown. They show a graded increase in the EPSP amplitude and a graded increase in the tension. *e*, The effect of 10^{-6} M octopamine in saline on the amplitude of IPSP (R5) elicited by stimulating the common inhibitory motoneurone. On the left a graded decline in the IPSP amplitude following superfusion with octopamine and on the right there is a graded recovery of the IPSP amplitude when the octopamine solution is replaced with saline. The oscilloscope is triggered by the stimulus delivered to the common inhibitor axon (S4) and five sweeps about 5 s apart are superimposed.

involved in synaptic modulation by our observation that the tension generated by the slow extensor motoneurone was potentiated by DUMETi stimulation (Fig. 3*a*) (M.O. and P.D.E. in preparation). The only mechanical effect of stimulating DUMETi alone is a small decrease in basal tonus. Exogenously applied octopamine enhances the tension evoked in the extensor muscle by the slow motoneurone (Fig. 3*c*) and this effect is blocked by the α -adrenergic blocking agent, phentolamine. The increase in tension ($<30\%$) is accompanied by a facilitation of the EPSP evoked by the slow motoneurone (Fig. 3*b-d*). In contrast, the IPSP elicited by stimulating the common inhibitory motoneurone is depressed by low concentrations of octopamine (Fig. 3*e*). These effects have a prolonged time course of many seconds. We could detect no effect of octopamine on the response of the muscle to stimulating the fast motoneurone.

We do not yet know the site of action or the mechanism of the synaptic modulation by octopamine. Octopamine could act on the postsynaptic muscle membrane or on the presynaptic terminals of the slow extensor and common inhibitory motoneurones and several mechanisms could mediate the effects shown here. Our demonstration, however, that octopamine can enhance excitation and depress

inhibitory neuromuscular synaptic transmission supports the growing evidence that amines have widespread modulatory roles^{7,20-22}.

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Magnetic ferrofluids for preparation of magnetic polymers and their application in affinity chromatography

THE use of magnetic polymers as supports for biological molecules has created much interest¹⁻⁵. The inherent advantages of such preparations are, in particular, the ease of recovery of these polymers by applying a magnetic field. Thus, when used as supports for immobilised enzymes, their easy retrieval from liquids containing colloids or undissolved solids should be of great practical value¹⁻³. Magnetic polymers have recently been tested as an alternative to conventional radioimmunoassay technique, obviating the need for vertical rotation and for the time-consuming, multiple centrifugations required with conventional solid phase procedures⁴⁻⁵. In the established techniques for producing magnetic polymer particles, the attachment of the biomolecules had to follow the preparation of the specific magnetic material; furthermore most of these preparations have been non-porous, exhibiting rather poor capacity. We describe here a novel and general procedure using magnetic fluids which allows 'post-magnetisation' of polymers already substituted with biological molecules. The properties of such preparations have been tested primarily as affinity chromatography gels. These preparations showed unaltered biospecificity when applied in general ligand-affinity chromatography studies. The simplified separation possible due to the magnetic properties of the gels eliminates the usual centrifugation and column chromatography steps. The procedure of 'post-magnetisation' seems to be suitable for different polymer particles, both unsubstituted gels and those carrying immobilised ligands as affinity adsorbents or enzymes.

The basic new principle in the procedure described here is the use of magnetic fluids. These can be characterised as fluids consisting of ultramicroscopic particles (~100 Å) of oxides. These are stabilised by a coating, typically 25-Å thick, preventing their sticking together in a magnetic field. Random collisions (Brownian motion) with the carrier

liquid's molecules keep the particles in colloidal suspension and are additionally stabilised by physicochemical means. In the following, ferrofluids, that is, those containing ferrite particles, have been used and with either water or hydrocarbon as the carrier. They behave like true homogeneous fluids, yet are highly susceptible to magnetic fields.

One gram of moist 5'-AMP- (or 2', 5'-ADP)-Sephacrose 4B (Pharmacia) which was previously swollen in 0.1 M sodium phosphate buffer pH 7.5, and washed with H₂O was packed in a column. Subsequently 6.5 ml of ferrofluid (base, H₂O; magnetic saturation, 200 G; trademark A05, from Ferrofluidics Corporation, Burlington, Mass.) were pumped through the column and cycled for 4 h at a flow rate of 50 ml h⁻¹ (usually at room temperature). After washing with 1 l of H₂O, on glass filter, incubation overnight at 4 °C with 100 mg of bovine serum albumin in 0.1 M Tris-HCl buffer pH 7.6, 5 mM in EDTA and 1 mM 2-mercaptoethanol was carried out, followed by washing with 200 ml 1 M NaCl and 200 ml of the same buffer. The dark brown gel beads were then magnetic and ready for use (Some enzymes tested are slowly inactivated, for reasons at present not yet definitely established, unless albumin treatment is carried out.)

When applying ferrofluids with a hydrocarbon as a base, an alternative procedure also involving albumin treatment was applied. The hydrocarbon-based ferrofluid (hydrocarbon; trademark HO1, magnetic saturation: 200 G) was circulated through the bed containing beads previously treated with solutions of gradually changing composition going from water to acetone. After the ferrofluid treatment the beads were filtered on a glass filter and washed with acetone. This was followed by successive washing with different solvent mixtures in the sequence: acetone, acetone-cyclohexane, cyclohexane, cyclohexane-acetone, acetone, water and finally buffer. To further increase the magnetic properties of the latter preparations they were treated once more before the final acetone washing. No differences in the affinity behaviour of the two types of preparations were noticed.

Whether the ferrite particles simply adsorb to the gel or precipitate out in the interior of the beads remains to be established. (In this context it should be noted that entrapment of ferrofluid within the lattice of polyacrylamide can easily be accomplished.) In any case, the magnetic properties of the gel remain unchanged after extensive washing with buffer (even washing with, for example, ethanol or 40% ethylene glycol did not remove the ferrite particles) and repeated use in magnetic fields; they are strong enough to allow rapid sedimentation even when applying weak permanent magnets.

To establish the usefulness of the magnetic Sepharose beads for affinity chromatography, we had to establish whether the biospecificity of the immobilised ligands remained unchanged, and to investigate the capacity of the gels. To study the former aspect, beef heart lactate dehydrogenase was chosen as model enzyme and applied to magnetised 5'-AMP-Sepharose 4B. As expected it bound to the 5'-AMP-gel, known to be specific for NAD⁺-dependent enzymes, and could subsequently be eluted quantitatively with 1 mM NADH (Fig. 1a). In contrast the NADP⁺-dependent enzyme 6-phosphogluconate dehydrogenase, chosen as reference, showed no affinity for the gel, which is in accord with previous findings using non-magnetised preparations of this general ligand⁶. Conversely, lactate dehydrogenase would not bind to 2', 5'-ADP, known to be specific for NADP⁺-dependent enzymes, in contrast to 6-phosphogluconate dehydrogenase, which bound and could be subsequently eluted with 1 mM NADPH (Fig. 1b).

To further investigate the usefulness of the magnetic affinity material prepared, isozyme separation of horse liver alcohol dehydrogenase was attempted. On applying a

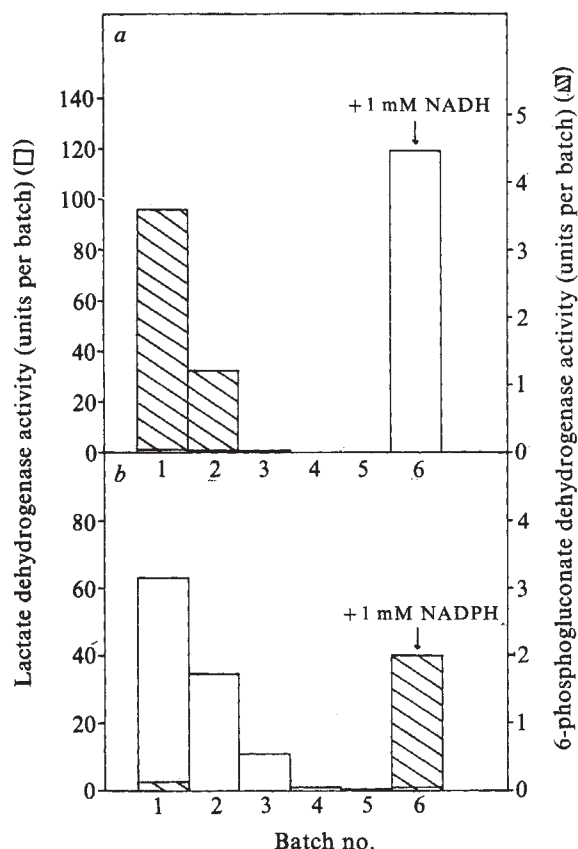


Fig. 1 *a*, To 0.4 g of moist magnetic 5'-AMP-Sepharose 4B (ferrofluid H_2O) were added 118 units (0.36 mg) of lactate dehydrogenase (from beef heart) or 4.5 units of phosphogluconate dehydrogenase (0.8 mg) from yeast in 2 ml of 0.1 M Tris-HCl, pH 7.6 containing 5 mM EDTA and 1 mM 2-mercaptoethanol. After incubation for 30 min on a rocking table (alternatively the gel could be kept suspended through a magnet kept at the outside of a rotating glass tube), the gel was settled in a beaker by applying at the outside a cylinder-formed permanent magnet with a pull of approximately 5 kg. After removal of the 'supernatant' from the gel (batch no. 1), the latter was washed 15 min in 4×10 ml of the incubation buffer (batch nos 2-5). Elution of the affinity-bound enzyme was achieved by incubation for 15 min in 10 ml of 1 mM NADH in the buffer. All experimental manipulations were carried out at 4 °C and enzymes assayed as described earlier⁶; batch no. on the abscissa refers to the number of successive washings and elution carried out with one affinity gel preparation. *b*, To 0.4 g of moist magnetic 2',5'-ADP-Sepharose 4B (ferrofluid, hydrocarbon) were added, as above, either lactate dehydrogenase or 6-phosphogluconate dehydrogenase. The experimental procedures were as for (a) except for the use of 1 mM NADPH.

mixture of the three isozyme forms EE, ES, and SS to magnetised 5'-AMP-Sepharose 4B, we found that they would adsorb to the gel. Afterwards a pulse of NAD^+ plus cholic acid eluted primarily the steroid active isozyme SS. With a subsequent application of NADH, the EE and ES forms were eluted, which is in analogy to previous findings⁷. It was found that the ratio of ethanol to steroid (5β -dihydrotestosterone) activity in the fractions eluted with NAD^+ plus cholic acid showed, after only one batch treatment, the low value of 1.3; this compares with 1.1 found for completely pure SS isozyme. (The applied mixture had a ratio of 7.) Clearly the elution technique used previously, which leads to purification of the SS isozyme, is applicable also to the magnetic polymer particles⁸.

Finally, a magnetised 5'-AMP-Sepharose 4B preparation was used for the purification of horse liver alcohol dehydrogenase from crude extracts⁷. As shown in Table 1, alcohol dehydrogenase of high specific activity was obtained in one step. The enzyme was almost pure as judged from sodium dodecylsulphate-gel electrophoresis. The procedure com-

Table 1 Purification of horse liver alcohol dehydrogenase by magnetic 'general ligand-affinity chromatography' in one step

Step	Specific activity (U mg ⁻¹)	Purification (n-fold)	Capacity (mg of protein per g moist gel)
Crude extract	0.08	1	
Magnetic 5'-AMP-Sepharose 4 B	1.70	21	0.7
5'-AMP-Sepharose 4B	1.98	25	1.5

A crude extract of horse liver was prepared as previously described⁷ with a protein concentration of 46.4 mg ml⁻¹. A large excess of protein (100-200 mg) in relation to 1 g of magnetic moist 5'-AMP (ferrofluid hydrocarbon)-Sepharose present in a beaker was applied. After incubation for 60 min on a rocking table, the gel was settled in a magnetic field and the 'supernatant' removed. Before elution of the affinity-bound enzyme, the gel was washed extensively with 0.1 M sodium phosphate buffer, pH 7.5 followed by a wash for 15 min in 0.1 mM NAD^+ (4 ml) and the above buffer (3×10 ml). The enzyme was then eluted by addition to the gel of 3 ml of 0.1 mM NAD^+ + 5 mM pyrazole in the above buffer (30 min, 4 °C). The lower set of values refers to a parallel run using identical amounts of non-magnetic affinity gel.

pared well with a blank run using non-magnetised 5'-AMP-Sepharose 4B, also in batch-fashion; only the capacity of the gel was diminished to about half. But its value of 0.7 mg of enzyme per g of moist gel is high when compared with some of the magnetic gels prepared in the beginning of this study following conventional approaches as entrapment of Fe_3O_4 particles inside gels.

A further advantage of the use of magnetic polymers in affinity chromatography lies in the fact that it allows a rapid 'pick-up' of molecules from colloidal solutions or those containing cell debris. Such quick retrieval would be most difficult or impossible to accomplish by conventional purification methods. This advantage seems to be of particular value if a rapid isolation of labile enzymes or enzyme complexes after their liberation from the cell is required. It is likely that once the enzyme is affinity-adsorbed within the pores of a gel, it is more protected, for example, against proteolytic enzymes. To test the ease of enzyme isolation, the following model experiment was carried out: horse liver was quickly ground and immediately thereafter magnetic 5'-AMP-Sepharose beads were added to the homogenate. After incubation, the cell debris was decanted by keeping the gel particles at the bottom of the beaker through a permanent magnet placed at the outside. After several batch-washings, alcohol dehydrogenase was ready to be eluted in NAD^+ plus pyrazole. Although the enzyme obtained showed lower specific activity (0.65 units mg⁻¹) than that given in Table 1, it clearly demonstrates the potential of the procedure.

Thus the simple technique applied here for the 'post-magnetisation' of pre-formed polymers already substituted with ligands has been shown not to alter the affinity properties of the polymers tested. This technique has also been applied successfully to unsubstituted gels such as Sepharose 4B or Sepharose Cl 6B. In addition, magnetic enzyme-bearing polymers were prepared applying the procedure described. Using trypsin as model enzyme, magnetic Sepharose-trypsin preparations were obtained with equivalent specific activities relative to soluble enzyme, as found with normal Sepharose-coupled trypsin preparations. In a typical experiment 5 mg of trypsin (bovine pancreas, Sigma type III) were allowed to couple to 1 g of moist Sepharose 4B activated by the CNBr procedure⁹. After washing, the Sepharose-trypsin preparation was treated for 4 h at 4 °C with 6 ml of ferrofluid (H_2O as base) in analogous manner to the procedure given.

Alternatively, the Sepharose beads were first magnetised and then underwent CNBr activation and trypsin coupling.

The latter preparation showed higher specific activity (relative to soluble enzyme) compared with the former (30% compared with 20%) but was somewhat less magnetic.

It seems that the magnetisation procedure described here using magnetic fluids will be of general interest, since in addition these fluids are also relatively inexpensive. We have demonstrated here the usefulness of the procedure for the preparation of suitable magnetic-affinity chromatography systems and for the preparation of magnetic enzyme-bearing polymers, the latter with potential in the area of enzyme technology. One can envisage other areas in which similarly prepared magnetic biomolecule-polymer complexes may find application, for instance as components of immunoassay systems; similarly magnetic carrier systems for 'targeting of drugs' could be designed carrying drugs, enzymes or other molecules of therapeutic value, which through a magnetic field can be brought to the desired site of action. In contrast to previous methods of preparing magnetic particles whereby in order to obtain the required magnetic properties one is forced into a choice of particular polymer, magnetic particle or procedure (often at the expense of other desired properties as porosity), such considerations are not involved in the simple procedure given here.

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Differential labelling of α and β -noradrenergic receptors in calf cerebellum membranes with ^3H -adrenaline

β -NORADRENERGIC receptor detection with radiolabelled catecholamines has been hampered by binding of catecholamines to other sites^{1,2}. Lefkowitz and Williams³ described binding of a synthetic catecholamine ^3H -hydroxybenzylisoprenaline to β -noradrenergic receptors on frog red blood cell membranes. Using procedures to prevent metabolism and nonspecific binding, we identified binding of ^3H -adrenaline to α -noradrenergic receptor sites in brain membranes^{4,5}. We report here the labelling of β -noradrenergic receptors in membranes of the calf cerebellum with ^3H -adrenaline.

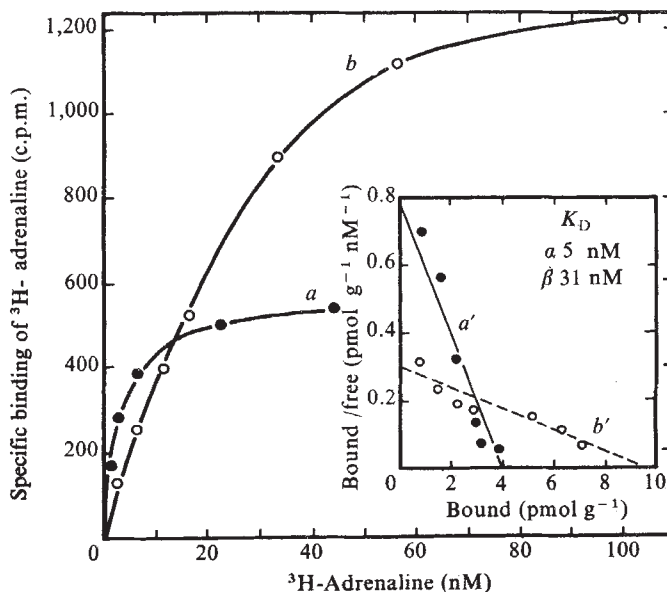
Homogenates of fresh or frozen calf cerebellar cortex were prepared as described previously⁴. Triplicate incubation tubes received 20 μl of diluted ^3H -adrenaline (DL-7, ^3H (N)-epinephrine L-bitartrate, 11 Ci mmol⁻¹, New England Nuclear) to give a final concentration of 10 nM ^3H -adrenaline, 10 μl of various concentrations of drugs, 20 μl of a solution containing 50 mM pyrocatechol, 5 mM disodium EDTA and 0.5 mM dithiothreitol, and 0.94 ml of tissue suspension. In β -receptor binding experiments, α -receptor binding was eliminated by the inclusion of 1.0 μM phentolamine in incubation solutions. Conversely, in α -receptor binding experiments, 1.0 μM ($\pm$)-propranolol was included to abolish β -receptor binding of ^3H -adrenaline. ^3H -Adrenaline and all other drugs were made up in 0.1%

ascorbic acid. The tubes were incubated at 25 °C for 60 min, and then rapidly filtered under vacuum through Whatman GF/B filters presoaked in 1 mM pyrocatechol. The filters were rinsed with 16 ml of ice-cold 50 mM Tris-HCl buffer containing 1 mM pyrocatechol and 0.1% ascorbic acid, and were subsequently counted by liquid scintillation spectrometry in 10 ml Formula 947 (New England Nuclear) at 37% efficiency.

Saturable or specific binding of ^3H -adrenaline to α -receptors was measured in the presence of 1.0 μM ($\pm$)-propranolol, as the excess over blanks which included 1.0 μM oxymetazoline. Saturable or specific β -receptor ^3H -adrenaline binding was measured, in the presence of 1.0 μM phentolamine, as the excess over blanks which included 1.0 μM ($\pm$)-propranolol. Previous experiments have demonstrated that after incubation of ^3H -adrenaline with calf brain membranes for 60 min at 25 °C in the conditions mentioned here, radioactivity eluted from the membrane pellet and radioactivity in the supernatant was essentially identical to cochromatographed stock ^3H -adrenaline⁵.

In the presence of 1 μM phentolamine, specific binding of ^3H -adrenaline to calf cerebellar membranes was saturable (Fig. 1). Scatchard analysis revealed a single population of binding sites, with a dissociation constant (K_D) of 31 nM. The specificity of ^3H -adrenaline binding in the presence of 1 μM phentolamine fulfilled characteristics expected of β -noradrenergic receptors. Of the principal catecholamines, ($-$)-isoprenaline was most potent in competing for binding (K_i = 1.9 nM), exceeding the potencies of ($-$)-adrenaline and ($-$)-noradrenaline by ten- and 100-fold respectively (Table 1). The 10-fold greater potency of adrenaline than of noradrenaline indicated that in calf cerebellar membranes, ^3H -adrenaline binding was associated with β_2 receptors. Binding of ^3H -adrenaline in the presence of 1 μM phentolamine was stereospecific,

Fig. 1 Specific ^3H -adrenaline binding to α - and β -receptors as a function of increasing concentrations of ^3H -adrenaline. Calf cerebellar homogenates (20 mg original wet weight of tissue) incubated for 60 min at 25 °C as described in the text, with various concentrations of ^3H -adrenaline. *a*, Binding to α -receptors, measured in the presence of 1.0 μM ($\pm$)-propranolol. Specific binding was that displaceable by 1.0 μM oxymetazoline. *b*, Binding to β -receptors, measured in the presence of 1.0 μM phentolamine. Specific binding was that displaceable by 1.0 μM ($\pm$)-propranolol. In each case, specific binding was defined as the difference between total and nonspecific. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times. *Inset*. Scatchard plots, showing K_D values of 5 nM at α -receptors, and 31 nM at β -receptors. Specific binding of ^3H -adrenaline to α -receptors at a concentration of 70 nM was not significantly different from that obtained at 40 nM ^3H -adrenaline, and is not shown in the concentration-binding curve, but is included in the Scatchard plot.



with (–)-catecholamines being 30–150 times more potent than the corresponding (+)-catecholamines. Affinities of other β -agonists paralleled their known pharmacological potencies and affinities for sites labelled by radioactive β -antagonists⁴. β -Noradrenergic antagonists competed potently and stereospecifically, with (–)-propranolol and (–)-alprenolol displaying apparent K_i values of 2–3 nM. Dichloroisoprenaline, (–)-practolol and butoxamine, pharmacologically weaker β -antagonists⁷ were comparably weaker in competing for binding of ³H-adrenaline. At concentrations as high as 10 μ M, drugs active at α -noradrenergic receptor sites failed to influence the binding of ³H-adrenaline, in the presence of 1 μ M phentolamine.

In the presence of 1 μ M ($\pm$)-propranolol, ³H-adrenaline seemed to bind selectively to α -receptor sites on calf cerebellar membranes, as reported earlier with calf cerebral cortex^{4,5} (Fig. 1, Table 1). In these conditions, the specific binding of ³H-adrenaline was saturable with maximal binding occurring by 20 nM. Scatchard analysis indicated a single population of binding sites, with a K_D value of 5 nM, similar to that found in the cerebral cortex. Thus ³H-adrenaline displayed about six times greater affinity for α - than for β -receptors. The substrate specificity of ³H-adrenaline binding in the presence of propranolol was as expected at α -receptor sites, with (–)-noradrenaline almost 100 times more potent than (–)-isoprenaline. The most potent catecholamine at apparent α -sites was (–)-adrenaline, with an affinity three times greater than (–)-noradrenaline. The α -agonist clonidine, the partial agonists ergotamine and piperoxan, and the α -antagonists phentolamine and WB-4101 were potent competitors for the binding of ³H-adrenaline in the presence of propranolol, with apparent K_i values in the 1–20-nM range. In general, the affinities of α -receptor agonists and antagonists in competing for ³H-adrenaline binding in the cerebellum in the presence of propranolol closely matched their affinities in competing for α -receptor binding by ³H-adrenaline, ³H-noradrenaline and ³H-clonidine in calf cerebral cortex^{4,5} and rat brain^{8,9}. Specificity of the binding of ³H-adrenaline to α -receptors in the presence of propranolol was supported by the very weak influence of β -agonists and β -antagonists on this binding (Table 1).

Kinetic studies were carried out to establish that the binding of ³H-adrenaline to both α - and β -receptor components was reversible. Specific binding in the presence of propranolol showed the same characteristics of association and dissociation at 25 °C as in the calf cerebral cortex⁵. Dissociation was biphasic, with a $t_{1/2}$ of about 1.5 min, slightly faster than in the cortex. Seventy to eighty per cent of specific binding was dissociated after 10 min. Association of ³H-adrenaline to β -receptors (in the presence of phentolamine) was more rapid than to α -receptors; equilibrium binding, attained in 15 min, remained stable for 2 h. A standard incubation time of 60 min was chosen to parallel conditions for measuring binding to α -receptors. Dissociation caused by adding a large excess of propranolol was extremely rapid at 25 °C, with a $t_{1/2}$ of about 20 s, and complete dissociation after 2 min.

In preliminary experiments, we failed to demonstrate binding of ³H-adrenaline to β -receptor sites in several rat brain regions, or in non-cerebellar areas of the calf brain. The ability to label β -receptors with ³H-adrenaline in calf cerebellum may be related to the greater density of β -noradrenergic receptors in this tissue detected by the binding of the β -antagonists, ³H-dihydroalprenolol and ¹²⁵I-hydroxybenzylpindolol (ref. 10 and our unpublished work), and to the observation that β -receptor sites in calf cerebellum are of the β_2 type, in which adrenaline is about 10 times more potent than noradrenaline.

Binding studies with a variety of neurotransmitter receptors in the brain have revealed distinct differences between interactions of agonists and antagonists^{8,11–15}. Most studies of β -receptors in various tissues have used only a radiolabelled antagonist. Evaluation of important questions of receptor functioning, such as desensitisation, requires studies of the

Table 1 Inhibition of ³H-adrenaline binding to α - and β -noradrenergic receptors in calf cerebellum

Drug	K_i at α -receptor (nM)	K_i at β -receptor (nM)
Catecholamines		
(–)-Adrenaline	2.4	21
(+)-Adrenaline		580
(–)-Noradrenaline	8	220
(+)-Noradrenaline		25,000
(–)-Isoprenaline	700	1.9
(+)-Isoprenaline		280
α-Agonists		
Clonidine	1.7	> 10,000
Oxymetazoline		> 10,000
α-Partial agonists		
Ergotamine	6	> 10,000
Piperoxan	13	
α-Antagonists		
Phentolamine	1.3	> 10,000
WB-4101	21	
β-Agonists		
Cc 25 (Desmethyl hydroxybenzylisoprenaline)		4
MJ 9184-1 (3-Methanesulphonamido-N-benzylisoprenaline)		6
Cc 34 (Hydroxybenzylisoprenaline)		25
Salbutamol	> 100,000	
Terbutaline	> 100,000	1,500
β-Antagonists		
(–)-Propranolol	23,000	2.3
(+)-Propranolol	8,000	60
(–)-Alprenolol	13,000	2.4
(+)-Alprenolol	7,000	23
Dichloroisoprenaline	20,000	730
(–)-Practolol	> 100,000	7,300
Butoxamine	> 100,000	12,000

Calf cerebellar cortex homogenates were incubated with 10 nM ³H-adrenaline for 60 min at 25 °C, in the presence of 1.0 μ M ($\pm$)-propranolol for α -receptor binding, or 1.0 μ M phentolamine for β -receptor binding, together with 3–6 concentrations of unlabelled drugs, using the standard assay conditions as described in the text. IC_{50} values were determined by log-probit analysis, and apparent K_i values calculated from the equation $K_i = IC_{50}/(1 + [^3H\text{-adrenaline}]/K_D)$. The K_D values used were 5 nM for α -receptor binding, and 31 nM for β -receptor binding. Values given are means from 2 to 4 experiments, each conducted in triplicate, which varied less than 20%.

direct binding of radiolabelled agonists. It is also desirable to label receptors with naturally-occurring transmitters or transmitter analogues. Accordingly, labelling β -receptors with ³H-adrenaline may facilitate an evaluation of receptor properties. Further studies now in progress show that in calf cerebellum the affinities of agonists are about 10-fold higher at sites labelled by ³H-adrenaline than at sites labelled by ³H-dihydroalprenolol, with the converse applying to antagonist affinities. These data suggest that a "two-state" model of receptor function may be applicable to the β -noradrenergic receptor as well as to the α -noradrenergic and other receptors in the central nervous system^{8,9}, although the differences between receptor interactions of agonists and antagonists are less marked at β -receptors than at other transmitter receptors. The possibility of labelling both α - and β -receptors in the same tissue with the same ³H-ligand may prove valuable in attempts to differentiate the properties of these two types of noradrenergic receptors.

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Purine and pyrimidine mononucleotides depolarise neurones of explanted amphibian sympathetic ganglia

ADENINE nucleotides and nucleosides have been proposed as neurotransmitters¹⁻³, in part based on their ability to inhibit spike activity in several peripheral^{1,4,5} and central^{2,5,6} sites. Only one preliminary study² has utilised intracellular recording to determine mechanisms of action of the purines in neurones. Because of their large size and simplified input-output relationships, neurones of sympathetic ganglia are a favourable model for intracellular recording. Although several studies have shown effects of ATP on peripheral ganglia^{7,8}, none utilised intracellular recording nor checked specificity of responses with other purine and pyrimidine

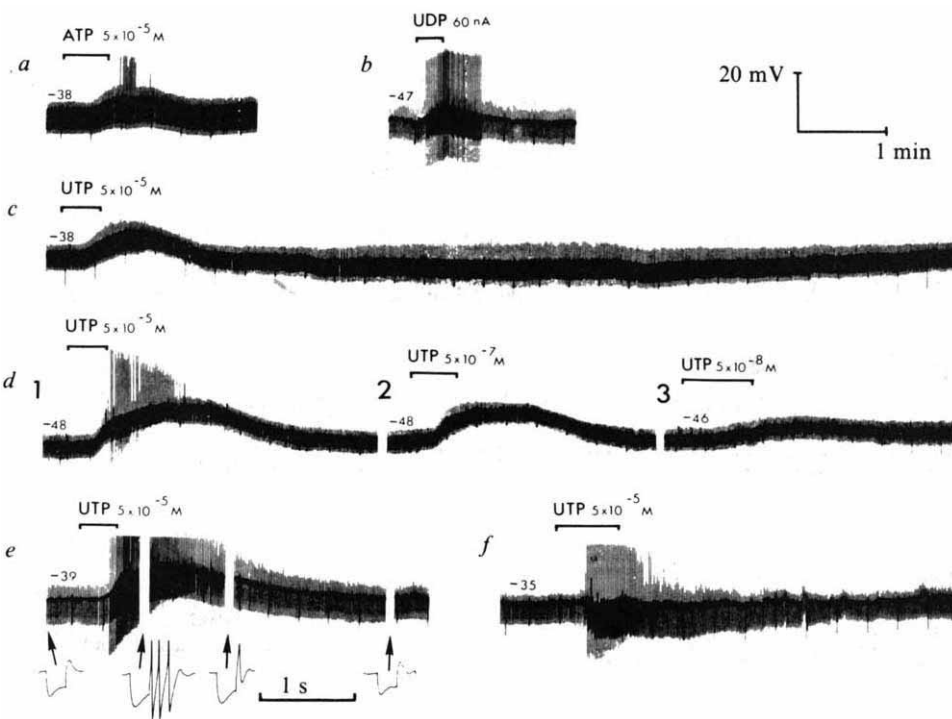
nucleic acid components. Therefore, we examined the effects of several purine and pyrimidine derivatives by intracellular recording of bullfrog sympathetic neurones in explant cultures. Our findings show pronounced depolarisations with the di- and triphosphoriboside derivatives of several pyrimidine and purine bases, with the uridine nucleotides emerging as the most potent of all the compounds.

We studied 182 sympathetic neurones, cultured from adult bullfrogs⁹ and impaled with glass micropipettes (3 M KCl or potassium acetate; 8-40 MΩ impedance) under Nomarski optics. Intracellular stimulation was carried out by a bridge method¹⁰ and current/voltage curves were constructed with a PDP-12 computer. The neurones studied ranged in size from 24 to 85 μm (long dimension) and displayed steady resting potentials of -35 to -58 mV with spike overshoots up to 30 mV or more. Drugs were applied by current-neutralised iontophoresis¹¹ or by rapid perfusion through a multiple valve system and a culture chamber with a volume of 0.3 ml.

Prolonged depolarisation was the predominant response to all the nucleic acid components studied, whether applied by perfusion or iontophoresis (Fig. 1a, b, d and e). Occasionally (14 of 98 cells) a long hyperpolarising component followed the depolarisations (Fig. 1c). In general, for any one purine or pyrimidine, the di- and tri-phosphonucleotides were equipotent as depolarising agents and more potent than their 5' monophosphonucleotides. The nucleosides guanosine, inosine and uridine and the heterocyclic bases guanine, adenine and uracil were ineffective at concentrations up to 1-2.5 mM. Adenosine depolarised six of eleven neurones weakly at 2.5-5 mM.

Of all nucleotides studied, 5' uridine di- and triphosphate (UDP and UTP) were the most potent with thresholds for

Fig. 1 Intracellular records of effects of mononucleotides on neurones cultured from the 8th-10th sympathetic ganglia of adult bullfrogs. Ganglia, cut into several pieces and placed on collagen coated coverslips, were cultured⁹ for 1-3 months at 22°C in the medium of McMahon and Kuffler¹⁴, also containing 0.3% methocel, streptomycin 100 μg ml⁻¹, glutamine 0.8 mM, and usually, NGF 1 U ml⁻¹. Heavy baselines in records show membrane potential (small numbers at beginning of each trace = mV). Light deflections below the baseline are responses to hyperpolarising constant current pulses of 0.05-1 nA and 200 ms duration, delivered through a Wheatstone bridge at 1 Hz. Examples of individual responses to current at higher polygraph speed below panel e. Pulses of 200 ms were used since this surpasses by 50-100 ms the initial unstable rectifying period typical of sympathetic neurones. Light lines above baseline are local depolarising responses to termination of current pulses (anodal break), which can trigger spikes. Heavy line descending below baseline every 20 s measures electrode resistance (1 MΩ/1 mV) by means of an RC circuit/ramp-input separate from the bridge circuit. a, Depolarising response to perfusion of ATP 5 × 10⁻⁵ M, with production of anodal break spikes. b, Extracellular iontophoresis of UDP (0.2 M pH 6-7 in pipette) by anodal current of 60 nA depolarises another neurone with spiking. c, Another neurone: perfusion of UTP 5 × 10⁻⁵ M causes depolarisation followed by a long hyperpolarisation; both phases accompanied by increases in input resistance, as assessed by size of hyperpolarising responses to constant current pulses, and increases in anodal break responses. d, Dose-related depolarisations of one neurone to UTP. e, Another cell: UTP 5 × 10⁻⁵ M depolarises cell with intense spiking; high speed excerpts taken from this record at times indicated by arrows show multiple anodal-break spikes and an increase in input resistance during depolarisation; bar below record indicates time for high speed records. f, Same cell as e: UTP causes anodal-break spiking even while membrane potential is held at resting level by injection of continuous hyperpolarising current; calibration bar in upper right corner applies to all records except high speed excerpts of panel e.



depolarisation of about 10^{-8} to 5×10^{-8} M; UTP 5×10^{-5} M produced depolarisations of 4–19 mV (mean = 10 mV; $n=40$). Dose-related responses are shown in Fig. 1d. Thresholds for 5' ATP and 5' ADP were about 10^{-6} to 10^{-7} M; ATP 5×10^{-5} M depolarised an average of 5 mV ($n=26$). Thymidine di- and triphosphate, guanosine di- and triphosphate and inosine di- and triphosphate were roughly equipotent with ATP. The uridine nucleotides seemed most potent also when applied by iontophoresis, producing depolarisations up to 16 mV; ATP and ADP evoked smaller depolarisations (up to 10 mV) with similar currents. Adenosine by iontophoresis was ineffective. Of all the nucleotides tested by perfusion, only 5' cytidine tri- and diphosphate were ineffective or only weakly active at 5×10^{-5} M. Pyrophosphate, 2' deoxy-ATP, uridine diphosphoglucose, and D-ribose 5' phosphate were largely ineffective at concentrations (5×10^{-5} to 10^{-4} M) which were maximally effective for the nucleotides.

Both the depolarisations and the occasional ensuing hyperpolarisations were generally accompanied by an increase (47 out of 65 cells) or no measurable change in input resistance, as determined either by measuring responses to hyperpolarising constant current pulses (Fig. 1e), or by comparing current/voltage curves (Fig. 2). Only five cells showed resistance decreases. Preliminary tests of voltage dependence showed that the size of the nucleotide depolarisation was inversely related to membrane potential (two cells) or was not affected (two cells) by changes in membrane potential from -70 to -6 mV.

Changes in spike generation (produced by intracellular stimulation) also occurred during the nucleotide responses. Most commonly, thresholds for spiking decreased (Fig. 1a, b, d, e and f) during the initial phases of depolarisation (40 out of 69 cells), although occasionally thresholds

increased during the later phases. A pure increase in spike threshold occurred in 10 of the 69 cells. The nucleotide-evoked increases in resistance and spiking are not dependent on the depolarisations, since they are not diminished by holding the membrane potential at resting level with current during perfusion of the mononucleotide (Fig. 1f). Further, depolarising the membrane by current to the same level as evoked by the nucleotide had no equivalent effect on spiking and resistance.

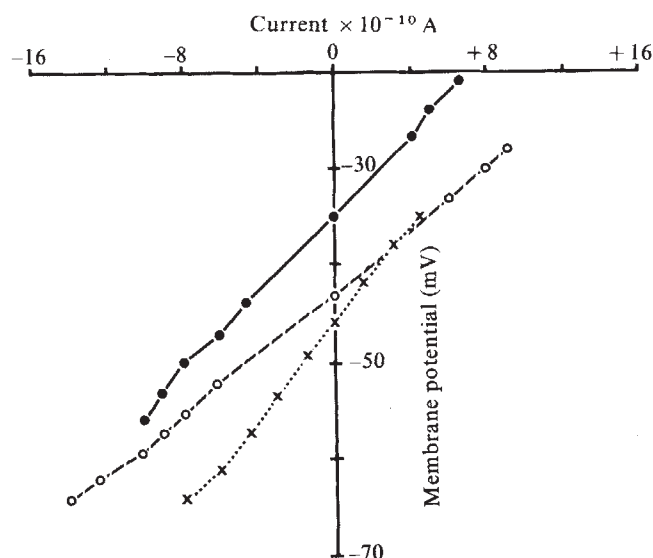
The mononucleotides act like the muscarinic cholinergic agent muscarine, which also depolarises cultured sympathetic neurones with increases in input resistance (G.R.S., D.L.G., A.L.P., and D.S.F. unpublished). The possibility that the nucleotides act by releasing endogenous acetylcholine, however, seems unlikely, since total blockade of the muscarine response by high concentrations of atropine (3×10^{-8} to 1.3×10^{-5} M) has no effect on the responses to the uridine or adenine mononucleotides (six out of six cells). Perfusion with nicotine 2.5 – 5×10^{-5} M (eight cells), (+)-tubocurarine 1 mM (four cells), or high Mg^{2+} (10–20 mM), low Ca^{2+} (0.5–1 mM) concentrations (six cells) also has little effect on the nucleotide responses. The fact that local application of UTP, UDP, ATP and ADP by iontophoresis markedly depolarised neurones, also argues for a direct effect of the nucleotides.

In preliminary studies, neither physostigmine (5×10^{-5} M), hexamethonium (0.5–2.5 mM), phentolamine (10^{-4} M) nor sotalol (1.5×10^{-4} M) altered responses to the nucleotides. Only quinidine (5×10^{-4}) blocked the UTP or ATP responses. This antagonism is, however, relatively unspecific, since the effects of muscarine are also blocked. The nucleotide receptor is not equivalent to the adenosine receptor of brain^{5,12}, since in our preparation methyl xanthines (2.5 mM) do not antagonise, but in fact occasionally potentiate responses to the mononucleotides. This might indicate mediation of the response by intracellular cyclic nucleotides. Perfusion of high concentrations of either cyclic AMP (10^{-3} – 10^{-2} M), cyclic GMP (5×10^{-3} M) or cyclic UMP (10^{-3} M), however, had only weak and variable effects on membrane potential, perhaps because of poor cell penetrability by the cyclic nucleotides. Finally, calcium chelation would not seem to be a factor in the nucleotide responses since such low effective concentrations of the nucleotides (10^{-7} – 10^{-8} M) are not likely to stoichiometrically remove significant Ca^{2+} from the Ringer's solution (containing 2 mM Ca^{2+}), and since Ca^{2+} removal does not reduce the responses.

The potent responses to several of the mononucleotides might indicate their function as neurotransmitters in so-called 'purinergic' nerves or as 'co-transmitters' which might be released with acetylcholine or noradrenaline from autonomic nerves. In our preparation, the generalised effectiveness of purine and pyrimidine mononucleotides suggests that the 'purinergic' label may be too restrictive, especially since the uridine nucleotides are the most potent. In the *Torpedo* electric organ cholinergic stimulation releases ATP postjunctionally¹³. Our results suggest that such release could modulate or even mediate the response to a neurotransmitter. Indeed, responses to the nucleotides bear a strong resemblance to the ganglionic late slow excitatory postsynaptic potential, which is also atropine resistant. Preliminary results show that iontophoresis of UDP and UTP increases firing rates of several types of rat central neurones *in situ* (G.R.S. unpublished). Taken with the present finding of a potent depolarising action of uridine nucleotides in cultured sympathetic neurones, these results suggest that the uridine nucleotides warrant close scrutiny for their possible involvement in neurotransmission.

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Fig. 2 Changes in current/voltage relationships of cultured sympathetic neurone in response to ADP. Curves were generated on-line by PDP-12 computer (DEC) using a modified ADTAPE program and FOCAL programs which select and average five consecutive digital points in equivalent control and pulse periods (200 ms duration) for current and voltage. During the current pulse, points were only sampled after the membrane potential had reached a steady level, generally 120–150 ms after pulse initiation (see high speed pulse records of Fig. 1e); care was taken to avoid selection of points where spikes occurred. ○, Control period; ●, peak of depolarisation to ADP 10^{-4} M; X, peak of subsequent hyperpolarisation to ADP (see text). Increase in slopes of the latter two lines indicate increase in input resistance during both the 8 mV depolarisation and the 2.5 mV hyperpolarisation to ADP. Control input resistance = 16 MΩ.



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Sodium channels in nerve apparently have two conductance states

THE inward sodium current underlying the nerve action potential is carried by discrete Na channels¹ in the membrane which are gated in response to the membrane potential. A basic question in understanding the gating mechanism is whether the conductance of the channels has only two states, open and closed, or whether there are more states, with the channel conductance progressing from one level to another to give the variable membrane conductance observed in voltage-clamp experiments. The ensemble fluctuation measurements reported here are consistent with the simple interpretation that Na channels have only two principal conductance states, with conductances of 0 and 7.7 ± 1 pS (near zero membrane potential), and number approximately 5×10^4 in the node of Ranvier of a 14 μ m diameter frog myelinated nerve fibre. Like stationary fluctuation analysis², the experimental method described here can yield information about the size and kinetics of the single gating events in ionic channels, but is also applicable to non-stationary processes such as membrane conductances that activate or inactivate with time. I report here measurements of the mean Na current and the variance at various times after the start of a depolarising pulse, using an ensemble of successively evoked current transients.

The preparation used was *Rana temporaria*, node of Ranvier; this is useful for fluctuation experiments because a stable voltage clamp with low background noise is readily obtained and because the number of Na channels is in a convenient range ($\lesssim 10^5$). Experiments were carried out at 2 °C to maximise the ratio of current fluctuations to thermal noise amplitude, and the voltage clamp electronics included a provision for measuring the membrane capacitance and the passive resistances of the intact preparation, allowing the thermal noise background to be estimated reliably. Potassium currents were blocked with internal Cs and external tetraethylammonium ions, and linear leak subtraction was used. A set of 64–160 Na current transients was elicited by repetitively depolarising the membrane at intervals of 300–500 ms. From groups of eight successive current records,

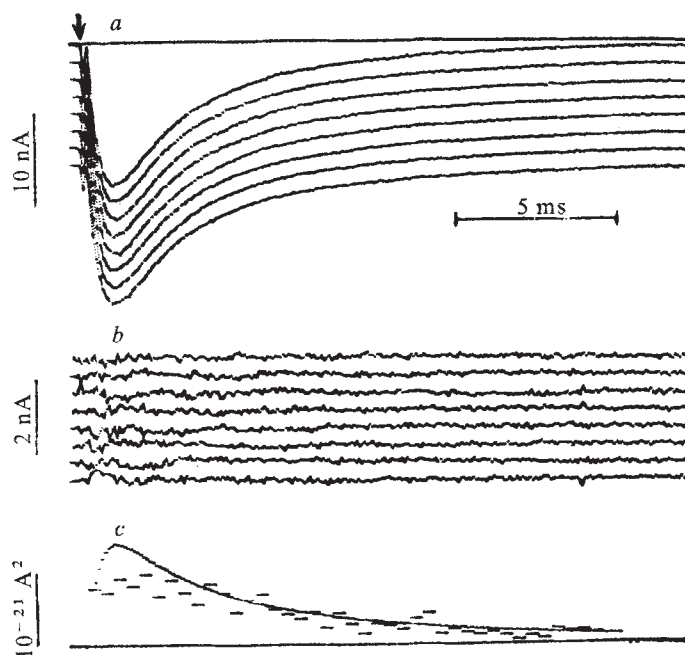


Fig. 1 Ensemble variance calculation. *a*, Eight current records aligned with the start of the depolarising pulse (arrow). *b*, Residual fluctuations from each record after subtracting the mean of eight. Node 8. *c*, Ensemble variance estimated from 72 records by the following process: the sum of the squares of the fluctuations was calculated for each time point; the resulting set of squared deviations was scaled and averaged with eight others; finally, the estimated background was subtracted. Each bar represents the mean of the resulting values at four adjacent time points. The smooth curve is the mean current record, scaled by a factor of -0.3 pA. Node 13. Bathing Ringer solution contained 20 mM TEA-Cl; cut internodes were in 110 mM CsCl, 10 mM NaCl. Current records low-pass filtered at 5 kHz (4 pole, Gaussian response) and digitised (12 bits, 256 samples per record) at 100 μ s intervals; aperture time jitter was < 50 ns. The thermal noise background was calculated (equations 11–16 of ref. 6) for each current sample, using the measured values for the internode and seal resistances and making use of the instantaneous I - V relation in estimating the membrane slope conductance. The peak value of the background component of the variance in *c* was 7×10^{-22} A².

mean and variance records were calculated point-by-point using a procedure similar to conventional signal averaging (Fig. 1). Several of these variance records were in turn averaged, and the conductance-dependent contribution from thermal and instrumental noise was calculated and subtracted, to yield an ensemble variance record. Figure 1*c* shows that the ensemble variance is proportional to the mean current at low conductance levels, but seems to saturate during the phase of high membrane conductance.

The contribution to the variance from processes other than channel gating was examined in several ways. First, the variance measured at V_0 , the reversal potential for Na current, was found to be accounted for (within an uncertainty of 2×10^{-22} A²) by the calculated thermal noise background. Secondly, long term changes in the amplitude of Na current were monitored and were found to be $< 2\%$ after 160 depolarisations in the experiments reported here. Because the mean and variance were calculated from small groups of successive records, linear drifts of that magnitude would not contribute significantly to the variance. Finally, the functional relationship between the variance and mean current values provides a clue to the source of the fluctuations. Any process which perturbs the properties of all the channels in the membrane coherently (for example, temperature or voltage fluctuations) would probably result in current fluctuations with variance increasing with the square of the mean current. Fluctuations in the transport of ions through

open channels would also be expected to give a monotonically increasing variance with increasing membrane conductance. Instead, the variance saturates and even decreases at high membrane conductance (Fig. 2), suggesting that neither of these processes is the predominant source of the fluctuations.

If the fluctuations were entirely due to Na channel gating, they could be interpreted on the basis of a simple open-closed scheme for the conductance of each channel. Assuming that the Na current is carried by a homogeneous population of N channels which are gated in a statistically independent fashion, the mean current I and the variance σ_i^2 would be given by³

$$I = Npi$$

$$\sigma_i^2 = Np(1-p)i^2$$

$$i = \gamma(V - V_0)$$

where i is the current carried by a single channel, γ is the single-channel conductance, V is the membrane potential and V_0 the reversal potential. The probability that a channel is in its open state is given by p , which is a function of both membrane potential and time. This theory assumes two conductance states but makes no assumption about the kinetics or voltage dependence of the gating process. A more specific theory based on the Hodgkin-Huxley equations would have $p = m^3h$.

The predictions of the two-state theory were tested in three ways. First, the relationship between I and σ_i^2 was observed as p changes with time during each depolarisation. Plots of these quantities (Fig. 2) were fitted with the predicted quadratic function, and data obtained with different depolarisations of the same node could be fitted with the same value of N . Second, the voltage dependence of the single-channel current i was compared with the 'instantaneous' current-voltage relation of the membrane as determined by a voltage-jump experiment (Fig. 3). The values of i (estimated by curve fitting as in Fig. 2) represent either the single-channel current of the two-state

Fig. 2 Variance plotted against the absolute value of mean current, with the time after the start of each depolarising pulse as the independent parameter. Points farthest from the origin correspond to the peak Na conductance. Depolarisations to -35 and 5 mV (node 14) and 125 mV (node 8), all with -105 mV, 50 ms prepulse from holding potential of -80 mV. Theoretical curves drawn with $N = 5 \times 10^4$; $i = -0.7$, -0.31 , and 0.2 pA, respectively.

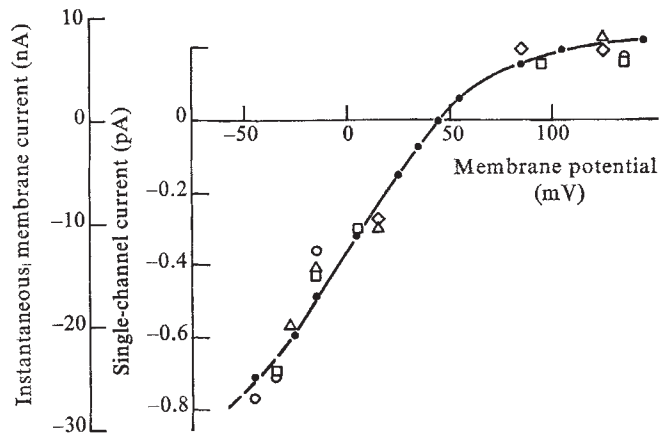
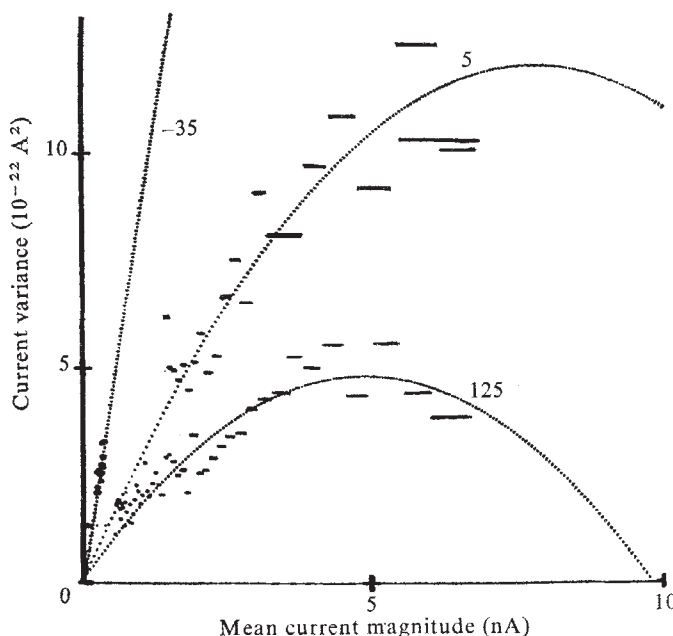


Fig. 3 Comparison of single channel currents and instantaneous I - V relation. Single channel currents from nodes $\diamond$, 8; $\circ$, 12; $\triangle$, 13; $\square$, 14. Curve follows instantaneous current values ($\bullet$) from node 8, measured at the potential indicated after a 2 ms prepulse to $V_0 = 44$ mV.

scheme or a weighted average of the various current levels in a multiple state scheme². The similarity in voltage dependence argues against multiple state schemes in which the distribution among states of different conductance is voltage dependent.

Third, values of γ were estimated under conditions which modify the peak Na conductance of the membrane. Pre-pulses in the range -105 to -55 mV changed the peak conductance by a factor of 4 but caused no significant change in γ . Treatment with 8 nM tetrodotoxin reduced the currents by a factor of 5 but also left γ unchanged. Changing the Na concentration or the pH of the solution bathing the node changed γ from the control value of 7.7 ± 1 pS measured at 0 mV in 90 mM Na to 4.1 ± 1.2 pS in 55 mM Na and to 2.5 ± 1 pS at pH = 5.2. Hydrogen ions are thought to alter the ion transport through open Na channels^{4,5}.

Although this estimate of γ is similar to another measurement from nodes of Ranvier⁶, it may have been affected by the presence of transport noise or excessive filtering. An upper limit on the effect of transport noise can be estimated if all of the channels are assumed to be open at the peak conductance at 125 mV (Fig. 2). The variance in the peak current would then be entirely from transport fluctuations in open channels. Assuming the transport noise variance increases linearly with the Na conductance, this excess variance would cause my estimate of γ to be higher than the actual value by at most 40%. Alternatively, γ would have been underestimated if a step in the gating process has a relaxation time ≤ 30 μ s, for part of the resulting fluctuations would have been filtered. The estimates of the total number of channels N were in the range of 3 – 6×10^4 , which corresponds to a Na channel density in the nodal membrane of roughly $1,000 \mu\text{m}^{-2}$. These values are tentative because the pool of channels that N represents may (because of ultraslow inactivation⁷, for example) be smaller than the total population in the membrane.

This work, like previous tests of the two-state theory⁶, has not ruled out all multiple conductance state schemes; there are theories with multiple conductance states which give predictions similar to those of the two-state theory. The simplest interpretation, however, is that Na channels have only two states of conductance.

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Modulation of electrical activity in *Aplysia* neurones by cholesterol

VARIOUS hormones and drugs induce long-lasting alterations of electrical activity in certain neurones from the marine snail *Aplysia californica*^{1–5}. Drugs and hormones were found to affect the lipid microviscosity of biological membranes^{6,7}, so it is plausible that their effect on electrical activity is mediated by changes in membrane fluidity. We report here that by changing the cholesterol content of *Aplysia* neurones, which presumably increases its membrane microviscosity⁸, long term, though reversible, alterations in electrical activity are induced.

In all experiments the visceral ganglion of *A. californica* was removed and the cell bodies were exposed by microdissection of the connective tissue. Microelectrodes filled with 3 M KCl were inserted into a neurone and its electrical activity was monitored using standard intracellular recording techniques⁹. Sonicated liposomes were used as exogenous pools for enrichment or depletion of the cell membrane cholesterol^{10,11}. For preparation of cholesterol-rich liposomes 160 mg of egg lecithin (Sigma type VE) and 100 mg of cholesterol (Sigma CHS) in chloroform were mixed in a sonication vessel and evaporated to complete dryness under argon. Ten ml of seawater was added and the mixture subjected to 70 W ultrasonic irradiation (Branson Sonifier W350) for 60 min at 0 °C in an argon atmosphere. The liposomal dispersion was then centrifuged at 60,000g for 20 min and the bottom and upper layers were discarded. The middle layer, which presumably consisted primarily of single walled liposomes¹², was used in the cholesterol enrichment experiments. For cholesterol depletion, liposomes of egg lecithin (160 mg in 10 ml seawater) were prepared analogously to the lecithin-cholesterol liposomes.

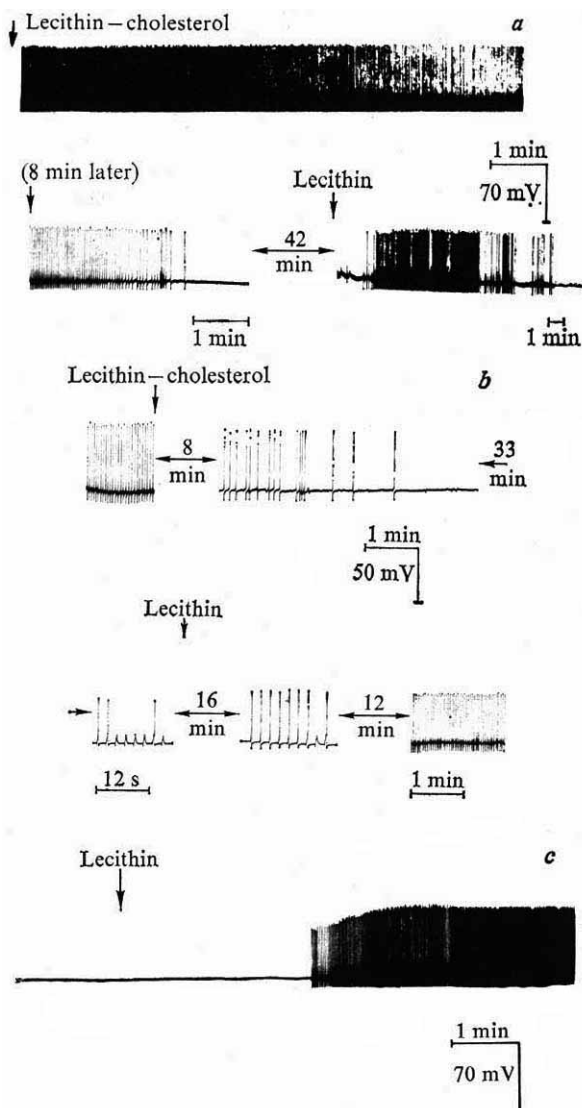
A freshly prepared liposome suspension was diluted 1:4 (v/v) in seawater and then applied to the ganglion by perfusion during continuous electrical recording for up to 3 h. In a series of 17 independent experiments perfusion of pacemaker and burster type neurones with lecithin-cholesterol liposomes was always found to gradually inhibit the spontaneous electrical activity of the neurones after a 4–10 min lag. After further 10–30 min all spontaneous activity ceased (Fig. 1a). Simultaneously, the threshold to elicit an action potential following intracellular stimulation progressively increased, in some cases reaching the stage where an action potential could not be produced by intracellular stimulation. Both the resting membrane potential and the steady-state slope conductance ($\Delta I/\Delta E$) of the *Aplysia* neurones were apparently not affected by the lecithin-cholesterol perfusion. When active neurones were perfused with lecithin liposomes, no decrease in activity was observed.

For estimation of the rate of cholesterol uptake analogous liposomes made from egg lecithin and ³H-cholesterol were prepared and applied separately to four isolated ganglia of similar size. The treated ganglia were thoroughly washed after 5, 15, 30, and 60 min of incubation, respectively, dissolved in a tissue solubiliser (NCS, Amersham) and then counted in a scintillation counter. Because of the large excess of the exogenous cholesterol in the incubation medium the rate of cholesterol depletion was considered as negligible compared to the rate of cholesterol uptake by the cells, which is therefore proportional to the accumulated radioactivity. The results showed a progressive uptake of cholesterol at a rate of approximately 300 pmol per h per ganglion. Assuming that the ganglion is composed of 2×10^3 cells averaging about 200 μ m in diameter¹³ which are partially exposed to the lecithin-cholesterol liposomes, and that the remaining glia cells do not interfere with the cholesterol uptake, then the observed rate of

cholesterol enrichment is of the same order of magnitude as in blood cells.¹⁰

The spontaneous electrical activity of the cholesterol-enriched neurones could be restored on subsequent perfusion with lecithin liposomes for less than 30 min (Fig. 1a,b). This treatment, which presumably depletes the membrane cholesterol in a similar fashion to that observed in other cells^{10,11}, demonstrates that the effect of cholesterol is reversible and passive. In some cases, the restored spontaneous electrical activity lasted until the electrode was withdrawn (over 1 h). In other experiments, however, spontaneous activity ceased after 10–20 min without affecting the capacity of the neurone to produce an action potential after intracellular stimulation. Furthermore, perfusion with lecithin liposomes of *Aplysia* neurones which were initially silent, generated continuous action potentials similar to pacemaker activity (Fig. 1c) which in most cases lasted until the experiment was terminated (over 1 h).

Fig. 1 Effect of liposome treatment on electrical activity of *Aplysia* neurones. *a*, Perfusion with lecithin-cholesterol liposomes ($4 + 2.5$ mg ml⁻¹ in seawater) (commenced at arrow) caused spontaneous activity to cease. The ganglion was then washed in seawater and perfused with lecithin liposomes (4 mg ml⁻¹ in seawater) which progressively restored electrical activity. The spontaneous activity was not maintained in this case. *b*, An identical experiment to (*a*) but with a separate ganglion. The response to a constant intracellular stimulation between the wash and after treatment with lecithin is shown. In this case, the restored spontaneous activity continued over 1 h when the electrode was withdrawn. *c*, Treatment with lecithin liposomes (4 mg ml⁻¹ in seawater) of an initially silent neurone. The activity continued until the experiment was terminated (over 1 h). All experiments were carried out at 25 °C.



One of the main factors which determine the lipid microviscosity of cell membranes is the mole ratio of cholesterol to phospholipids (C/PL). In *in vitro* conditions this determinant can be modulated by treatment with liposomes by two main mechanisms. First, in the translocation mechanism, cholesterol molecules partition between the lipid pools of the liposomes and the membrane towards equilibration of their C/PL levels. This takes place in treatment of erythrocytes¹⁰, lymphocytes¹¹ and platelets¹⁴ with liposomes of various C/PL. Second, the fusion mechanism, involves integration of the liposomes by fusion with the cell membrane¹⁵, presumably through specific receptors¹⁶. The fusion mechanism was demonstrated in a series of cells including lymphocytes¹⁷ and fibroblasts¹⁸. In general, these two mechanisms do not affect the viability of cells and eventually lead to a similar change in C/PL of the cell membrane. Because of technical difficulties, we have been able to establish which mechanism operates in the perfusion of the *Aplysia* neurones with liposomes.

The common methods for measurements of membrane microviscosity⁸ could not be applied to the isolated *Aplysia* ganglia. But, it is reasonable to assume that, as for most other cell membranes, enrichment with cholesterol markedly increases the microviscosity⁸, as well as the degree of exposure of membrane proteins¹⁹, and decreases the turnover of individual membrane enzymes and transport channels. The net effect of these modulations is expressed in alterations of cellular activity²⁰. The findings presented here suggest that the activity of membrane sites, which are associated with spontaneous electrical activity and generation of action potential, is also dependent on membrane fluidity as determined by the cholesterol level. In principle, decrease in membrane fluidity can also be achieved by reducing the temperature. It has been previously shown² that by lowering temperature, the activity of *Aplysia* neurones is reduced, but to a lesser extent than with cholesterol enrichment. Possible alteration of the Na, K ATPase activity in the cholesterol enriched membrane^{21,22} could be one of the factors which mediate the observed changes in electrical activity. As the resting membrane potential was not affected by the cholesterol enrichment or depletion, however, it seems that the electrogenic Na, K pump, which exerts a tonic contribution to the membrane potential in *Aplysia* neurones²³, is not noticeably affected by membrane fluidity. Alteration in internal Ca activity could also contribute to the observed effects^{24,25}. The lack of substantial changes in conductance and resting membrane potential upon enrichment with cholesterol suggests, however, that the major effect is on the channels and gates which are essential for action potential production²⁶. Since the action potential height decreases during cholesterol treatment (Fig. 1b), and in some cases after prolonged perfusion only a small spread-out regenerative response could be elicited, it seems that inclusion of cholesterol decreases the active inward conductance. For a better understanding of the effect described here, voltage clamp and ion selectivity experiments are required.

The microviscosity of biological membranes may vary in various physiological and pathological conditions²⁷. Neuroblastoma cells have been shown to undergo a marked modulation in membrane microviscosity during their cell cycle²⁸. On differentiation of these cells the membrane fluidity is increased and this process can therefore be blocked by cholesterol enrichment²⁹. By changing the lipid composition of neuroblastoma cells conspicuous morphological changes have been also observed³⁰. Such changes may also occur in other nerve tissue, and may induce a significant alteration of neuronal activity *in vivo*.

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Asymmetry of phospholipid biosynthesis

THE asymmetrical distribution of phospholipids in the membrane of the red blood cell^{1–3} and other membranes^{4,5} is well documented. How this asymmetry arises in these membranes is an obvious question which remains unanswered. One explanation is that the enzymes that catalyse the last step in the synthesis of the phospholipids are unevenly distributed on both sides of the membrane where biosynthesis occurs. The phospholipids that are produced by these enzymes would be released on to each side of the lipid bilayer and thus account for lipid asymmetry. A necessary corollary would be that this asymmetry of the phospholipids would be preserved in the movement of lipids to other membranes in the cell. Initial reports on the lack of transbilayer movement of phospholipids in artificial membranes^{6,7} and membranes of animal viruses⁸ has made an uneven distribution of phospholipid biosynthetic enzymes an attractive hypothesis. Recent reports, however, have documented the transbilayer movement of phospholipids in the red blood cell membrane^{9–11} and the membrane of *Bacillus megaterium*¹². Although the mechanism for this transbilayer movement of phospholipids has yet to be elucidated, it is no longer necessary to explain lipid asymmetry by the uneven distribution of the phospholipid biosynthetic enzymes on both sides of the membrane. We summarise here our evidence that the enzymes that help to produce phosphatidylcholine and phosphatidylethanolamine are located exclusively on the cytoplasmic surface of the endoplasmic reticulum of rat liver and not on both sides of the membrane.

Subcellular fractionation of rat liver yields a microsomal fraction which consists largely of sealed fragments from the endoplasmic reticulum¹³. These vesicles retain the asymmetry of the endoplasmic reticulum as it exists in the cell; the exterior of the microsomal vesicles corresponds to the cytoplasmic surface of the endoplasmic reticulum, whereas the luminal surface is the interior of the microsomes¹³. Preparation of microsomes with the opposite orientation has not been reported.

A rapid method for evaluation of the permeability and orientation of microsomes by measurement of the latency of mannose-6-phosphatase has been reported^{14,15}. The enzyme that hydrolyses mannose-6-phosphate is located inside the microsomes, which are apparently impermeable to low concentrations (1 mM) of this substrate. Treatment of microsomes with 0.4% taurocholate allows the substrate to penetrate the vesicles¹⁴. Thus, by assay of the mannose-6-phosphatase before and after treatment with taurocholate, one can assess the orientation and intactness of the membrane of the microsomal vesicles. The latency of this enzymatic activity is defined as the "activity in disrupted microsomes minus activity in untreated microsomes/activity in disrupted microsomes"¹⁵.

Microsomes from rat liver were prepared as described by Nordlie and Arion¹⁶, except that homogenisation was in 0.145 M NaCl and contaminating cytoplasmic enzymes were removed by passage of the microsomes once through a discontinuous sucrose gradient¹⁷. The microsomes were suspended in 0.145 M NaCl at approximately 7 mg protein ml⁻¹ and stored at -70 °C. The latency of mannose-6-phosphatase in all preparations was greater than 90%. The activities of CDP-choline: 1,2-diacylglycerol cholinephosphotransferase and CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase were assayed essentially as described by Kanoh and Ohno¹⁸. The assays of cholinephosphate cytidyltransferase¹⁹ and phosphatidylethanolamine-S-adenosylmethionine methyltransferase²⁰ followed published procedures.

In our initial experiments with the cholinephosphotransferase (which condenses CDP-choline and 1,2-diacylglycerol to form phosphatidylcholine), we found that treatment of the microsomes with 0.4% taurocholate resulted in a sixfold increase in the cholinephosphotransferase activity (from 3.3 to 19 nmol per min per mg protein). We did not know if this increase in enzyme activity were due to stimulation by the detergent or the expression of latent activity localised on the interior of the microsomes.

One approach for differentiation between these two possibilities was to digest the microsomes with a proteolytic enzyme and subsequently treat the microsomes with 0.4% taurocholate. Cholinephosphotransferase activity localised on the interior of the microsomes should survive this treatment. Rat liver microsomes (1.4 mg protein) were digested with bovine pancreatic trypsin (1 mg; Sigma), in 0.5 ml 50 mM Tris-HCl, pH 7.4, for 15 min at 30 °C. The reaction was stopped with the addition of 2 mg soybean trypsin inhibitor (Sigma). In control incubations the trypsin inhibitor was added before the trypsin, or isotonic saline was added instead of trypsin and trypsin inhibitor. Samples (0.18 ml) were removed and incubated with 20 µl 4% sodium taurocholate dissolved in 50 mM Tris-HCl, pH 7.4, or with buffer alone, for 30 min at 0 °C. The activity of cholinephosphotransferase was subsequently assayed. By this treatment the activity of the enzyme decreased from 19 to 0.5 nmol per min per mg protein. Since more than 97% of the cholinephosphotransferase was inactivated by trypsin, it seemed that none of the cholinephosphotransferase was localised on the interior of the microsomes.

It was important for us to demonstrate that during the trypsin digestion, the microsomes remained intact and impermeable to the trypsin. We therefore carried out an experiment in which the activity of the cholinephosphotransferase and the latency of the mannose-6-phosphatase were determined after incubation with trypsin for various times. The results in Fig. 1 clearly show that the latency of the mannose-6-phosphatase was not affected by the proteolytic digestion, whereas the activity of the cholinephosphotransferase was eliminated. Other studies have shown that the mannose-6-phosphatase is inactivated by trypsin in the presence of 0.4% taurocholate.

Similar studies were performed with CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase (which forms phosphatidylethanolamine), cholinephosphate cytidyltransferase (which forms CDP-choline) and phosphatidylethanolamine methyltransferase (which transforms phosphatidylethanolamine to phosphatidylcholine by the transfer of methyl groups from S-adenosylmethionine). The experiment in Fig. 1 shows that these enzymes are also inactivated by trypsin in conditions in which mannose-6-phosphatase activity is unaffected. It seems, therefore, that the enzymes that synthesise phosphatidylcholine and phosphatidylethanolamine are localised on the exterior surface of the microsomes.

There is an additional explanation of our results which should be considered. It is possible that the phospholipid biosynthetic enzymes extend across the bilayer of the microsomal membrane. Tryptic digestion of the part of the protein that is exposed to the exterior might inactivate the enzyme even if the active site were on the inside of the microsomal membrane. We are unaware of any example where there is this arrangement of a membrane-bound enzyme. Moreover, if the active site were on the interior of the microsomes, transport of the substrate into the microsomes must

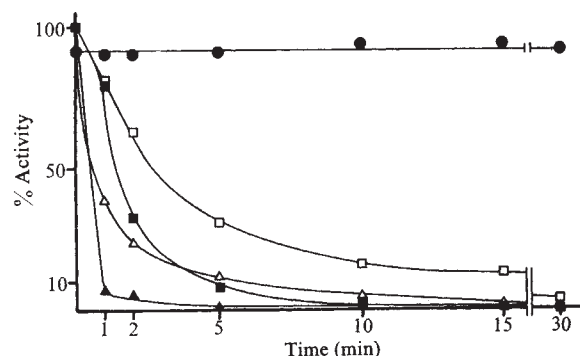


Fig. 1 Effect of trypsin on the activity of phospholipid biosynthetic enzymes associated with microsomes from rat liver. Microsomes (5 mg) were incubated with 3.5 mg trypsin, 10 mM Tris-HCl, pH 7.4, at 30 °C for the indicated time. Soybean trypsin inhibitor (5 mg) was added to terminate the reaction (final volume 1.5 ml). Aliquots from each incubation mixture were used to assay for cholinephosphotransferase¹⁸ (■), ethanolaminephosphotransferase¹⁸ (□), cholinephosphate cytidyltransferase¹⁹ (▲) and phosphatidylethanolamine methyltransferase²⁰ (Δ) activities. Intactness of the microsomes was determined by measurement of the latency of mannose-6-phosphatase^{14,15} (●).

occur. We have been unable to detect transport into the microsomes of any of the substrates for these phospholipid biosynthetic enzymes.

We conclude from our experiments that the enzymes that synthesise phosphatidylcholine and phosphatidylethanolamine are located on the cytoplasmic side of the endoplasmic reticulum. Thus, the origin of lipid asymmetry does not result from an uneven distribution of these enzymes on both sides of the endoplasmic reticulum. It remains a moot question as to how lipid asymmetry originates and is maintained in biological membranes.

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Light-sensitive membrane potentials in onion guard cells

INTRACELLULAR electrical recordings in onion guard cells show that they maintain a membrane potential difference (MPD), inside negative. The MPD is light-sensitive; cells subjected to short light and dark cycles depolarise in the dark and hyperpolarise in the light. The swiftness of the electrical changes makes them among the fastest known stomatal responses, suggesting a causal relationship between the reception of light, the changes in membrane potential and the ion fluxes known to be associated with stomatal movement. The magnitude and electrical sign of the responses are consistent with the activity of a light-sensitive proton pump which would provide the driving force for ion transport across the cell membrane. Guard cells, the specialised structures that control gas exchange in the leaves of higher plants, use potassium to modulate their intracellular osmotic potential and perform the mechanical work needed to open and close the stomatal pore¹. When guard cells accumulate K⁺ (with intracellular concentrations reaching up to 0.5 M (refs 2 and 3)), water content increases and swelling occurs, causing the pore to open. During the reverse process, K⁺ is extruded (intracellular concentration diminishes to 0.1 M (refs 2 and 3)), leading to water loss and pore closure. Control of intracellular K⁺ concentrations is a universal feature of living organisms. Nerve cells use energy used to generate electrical signals by maintaining concentration gradients of Na⁺ and K⁺ (ref. 4); bacterial cells selectively accumulate K⁺ (ref. 5) and, in plants, cells expand by increasing their turgor through uptake of K⁺ and its counter-ions⁶. While the cellular basis of K⁺ uptake in stomatal guard cells is poorly understood, one might expect common mechanisms for K⁺ transport even in widely divergent species. Two distinct mechanisms for K⁺ transport have been defined. In one, common in animal cells, K⁺ uptake is driven by ATP through a Na⁺/K⁺ ATPase⁷; in the second system, demonstrated in bacteria, ion transport is conducted by a proton-motive force⁸ generated by pH and electrical potential gradients across the membrane⁹. In showing that wall-less guard cell protoplasts swell when illuminated, we previously argued that a proton-motive force could drive K⁺ uptake into the guard cells¹⁰. The hypothesis generates the basic prediction of a light-sensitive, inside negative, MPD across the guard cell membrane. We report here the experimental verification of that prediction, through intracellular recordings in guard cells of *Allium cepa*.

Both intact guard cells and cells with walls partially digested with a cellulolytic enzyme were used. Partial digestion of cells facilitated electrode penetration and gave us a basis for work on MPD and cell compartments, which are discussed elsewhere¹¹. Slices from cotyledons of 7–14 d-old seedlings were the tissue source for both types of preparations¹². The slices were adhered with a Valap mixture¹² to the bottom of a 5 ml well made in a 9.5-cm Petri dish filled with Sylgard (Dow Corning) and bathed with a 0.23 M mannitol solution containing 1 mM KCl and 1 mM CaCl₂. The wall digestion was made with a 2% (w/v) solution of Cellulysin (Calbiochem) in the above solution for 2–5 h. At the end of the digestion, the preparation was washed twice with enzyme-free, bathing solution. Both types of preparations were dark adapted for 1–4 h before the experiments. Guard cells were impaled with glass capillary microelectrodes under direct visualisation at 400× magnification using a Zeiss compound microscope with a water-immersion objective. Microelectrodes were filled with 3 M KCl and bevelled after filling to final resistances of 40–90 MΩ. The signal from the microelectrode was put

through a high input impedance preamplifier and displayed on a cathode-ray oscilloscope and a slow speed chart recorder.

All guard cells showed MPD, inside negative. Partially digested cells averaged -39 ± 7 mV ($n=65$) and intact guard cells -72 ± 29 mV ($n=40$). Exposure of the cells to light cycles showed that the MPD is light-sensitive. Guard cells were impaled under dim green light, and those showing steady MPD (± 2 mV) for at least 5 min, were subjected to alternating short dark and white light (200–500 $\mu\text{E m}^{-2} \text{s}^{-1}$) cycles. The cells typically depolarised in the dark and hyperpolarised in the light (Fig. 1). Both intact and partially digested cells exhibited the electrical changes but the former had faster and larger responses. Depolarisations ranged between 1 and 60 mV; hyperpolarisations between 1 and 52 mV. The initial stages of the responses showed the highest rates, reaching as much as 1.7 mV s^{-1} for the depolarisations and 0.9 mV s^{-1} for the hyperpolarisations. Initial rates varied greatly, though, averaging about 14 mV min^{-1} for depolarisations and 8 mV min^{-1} for hyperpolarisations. After an initial fast phase, the responses become slower, saturating with approximately exponential kinetics (Fig. 1b). In addition, prolonged depolarisations showed a second, slow phase (Fig. 1b) which could not be observed in the hyperpolarisations. Actively responding cells showed alternating responses throughout 3–5 consecutive light/dark cycles with latencies ranging from 1–45 s (Fig. 1a). Typically, the depolarisations had shorter latencies.

It seems likely that these electrical responses of the guard cells are normal physiological events, associated with the K⁺ fluxes seen during stomatal opening and closing. The short latencies and high initial rates of these light-induced membrane potential changes suggest that they could be an early and primary event in the mechanism controlling stomatal movement. The fact that the latencies of the dark responses, which we interpret as related to stomatal closing, are of the same order of magnitude as the latencies reported for closing in response to high CO₂ concentrations¹³ also suggest a primary effect of light. The present results argue against the possibility that stomatal light responses are indirect and CO₂-mediated¹⁴, since it is difficult to envisage that dark would cause a CO₂-mediated response with the same latency as the CO₂ response alone. The data suggest, instead, that both dark and CO₂ can elicit a primary response leading to closing. Our observations showing that isolated guard cells from digested preparations also exhibit light-sensitive MPD indicate that light energy was absorbed by photopigments within the guard cell, without the mediation of other cells of the tissue.

An electrogenic mechanism driving K⁺ transport in the guard cells has been suggested before¹ but the hypothesis has not become widely accepted because of negative reports on MPD across the guard-cell membrane³ or its sensitivity to light¹⁵. The data presented here and related observations by Gunar *et al.*¹⁶ give new support to the notion of an electrogenic pump being the driving mechanism of stomatal movement.

Light-induced electrical events associated with pump activity are well known in other systems. Vertebrate photoreceptors hyperpolarise when illuminated due to a change in membrane Na⁺ conductance, probably mediated by the release of Ca²⁺ from intracellular stores¹⁷. It is of interest that both onion guard-cell protoplasts¹⁰ and outer-rod segments isolated from frog retinae¹⁸ change their intracellular osmotic potentials in response to light. It seems plausible that the two cell types might share common mechanisms underlying phototransduction and ion fluxes.

Another useful system which provides insight on the basis of light-driven energy transduction coupled to ion transport has been described in *Halobacterium halobium*. In

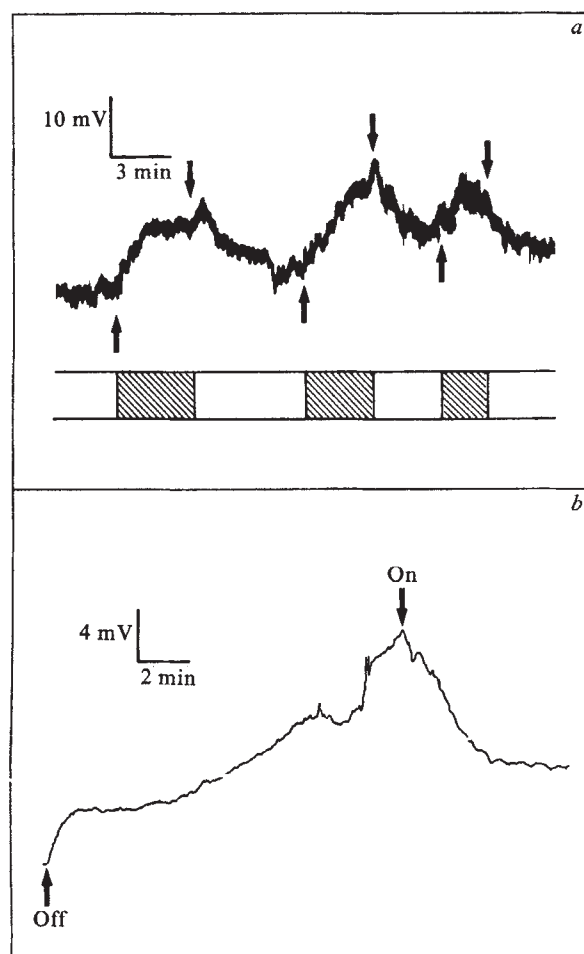


Fig. 1 Intracellular electrical recording in onion guard cells showing the changes in membrane potential differences in response to light and dark cycles. *a*, Three alternating dark/light cycles in an intact cell. Shaded portions in the abscissa show the dark periods, arrows point to the onset of each alternating stimulus. *b*, Dark and light response in a partially digested cell, seen at a higher gain. Both depolarisations and hyperpolarisations saturate with apparent exponential kinetics, with the depolarisations showing a second slower phase.

this microorganism a membrane-bound photopigment, bacteriorhodopsin, extrudes protons when illuminated, thus generating an electrochemical gradient across the cell membrane. This proton motive force can then be used to drive passive K^+ influx¹⁹ or to make ATP (ref. 20). In addition, ATP hydrolysis can be used to poise the proton gradient independently of illumination²⁰, thus making the gradient a central device for the transduction of energy from either light or respiration²¹. Light-induced membrane hyperpolarisations ranging between 24–67 mV have been measured in *Halobacterium*²².

The demonstrated ability of a proton gradient to drive K^+ uptake in the light and in the dark makes it a possible mechanism for energising ion transport during stomatal movement. Most of the known physiological properties of the guard cells seem consistent with the hypothesis and our findings of a light-sensitive MPD in onion guard cells adds new, though indirect, evidence.

The demonstration of a proton gradient across the guard-cell membrane as a primary energy-transducing mechanism would provide a link between the several possible energy sources used by the guard cells to drive ion fluxes. Blue light energy could be directly transduced as an electron flow when absorbed by a photoreceptor (a flavin?) which would be one of the links in the membrane-bound, electron transport chain which generates the

gradient. In addition, ATP hydrolysis would constitute a major energy source poising the gradient. ATP could be formed by oxidative phosphorylation in the guard cell mitochondria, which would be light independent and able to drive ion fluxes in the dark or by photophosphorylation in the guard cell chloroplasts which could utilise light energy with the spectral sensitivity of chlorophyll.

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Unprotonated chromophore-protein bond in visual pigments from ¹³C-NMR spectra

THE chromophore of visual pigments is generally assumed to be a protonated Schiff base of the 11-*cis* retinylidene (rhodopsins) or the 11-*cis* 3-dehydroretinylidene (porphyropsins) groups linked through a lysine amino group to a specific glycolipoprotein moiety (opsin)¹. This protonated Schiff base hypothesis has been supported by plausible though not unequivocal chemical evidence^{2,3}. Recent studies using the physical technique of resonance Raman spectroscopy have also seemed to support this view^{4–7}. In an attempt to confirm this assignment and to explore further the structure and microenvironment of the chromophore, we have studied the nuclear magnetic resonance (NMR) spectra of aqueous suspensions of bovine rhodopsin which contain the chromophore enriched with ¹³C at specific positions in the retinylidene carbon chain. We report here our findings which clearly support an unprotonated retinylidene Schiff base as the visual chromophore in contrast to the earlier chemical and physical evidence which supports a protonated form.

Retinal isomers were synthesised enriched at the C14 position

from the C18 ketone⁸ and triethyl phosphono[2-¹³C]acetate⁹. This latter compound was synthesised from triethylphosphite and ethyl [2-¹³C] bromoacetate. The resulting ester was converted to the [14-¹³C] retinal isomers by LiAlH₄ reduction and MnO₂ oxidation. High pressure liquid chromatography was used to isolate the 11-*cis* isomer of [14-¹³C] retinal¹⁰. ¹³C-labelled rhodopsin was prepared by regenerating freshly photobleached bovine disks with labelled 11-*cis* retinal. After adding hydroxylamine to complex the excess retinal, the rhodopsin was solubilised by adding 50 mM aqueous phosphate-buffered octyl glucoside. Purification and delipidation was accomplished by affinity chromatography on Concanavalin A-Sepharose^{11,12} using 30 mM octyl glucoside as detergent. This solution was then concentrated to 1–2 mM and dialysed three times to remove small organic molecules. Nuclear magnetic resonance

(NMR) spectra were collected in the Fourier transform mode on a 14 kG home built (DT) spectrometer as well as a Varian XL-100.

Figure 1a shows the ¹³C NMR spectrum of a solution of octyl glucoside-solubilised bovine [14(retinylidene)-¹³C] rhodopsin containing no free retinal isomers. Figure 1b shows a spectrum of a solution of octyl glucoside and unenriched rhodopsin. The spectrum of the enriched pigment contains one additional resonance at approximately 130.8 p.p.m. downfield from TSP-d₄. This peak is more pronounced in Fig. 2, where the rhodopsin concentration is twice that in Fig. 1a and the ¹³C-¹H dipolar interactions have been removed with high power decoupling. The peak at 175 p.p.m. in Fig. 2 is due to resonances arising from the amide carbonyls of the protein, observed because of the long pulse delay in this experiment.

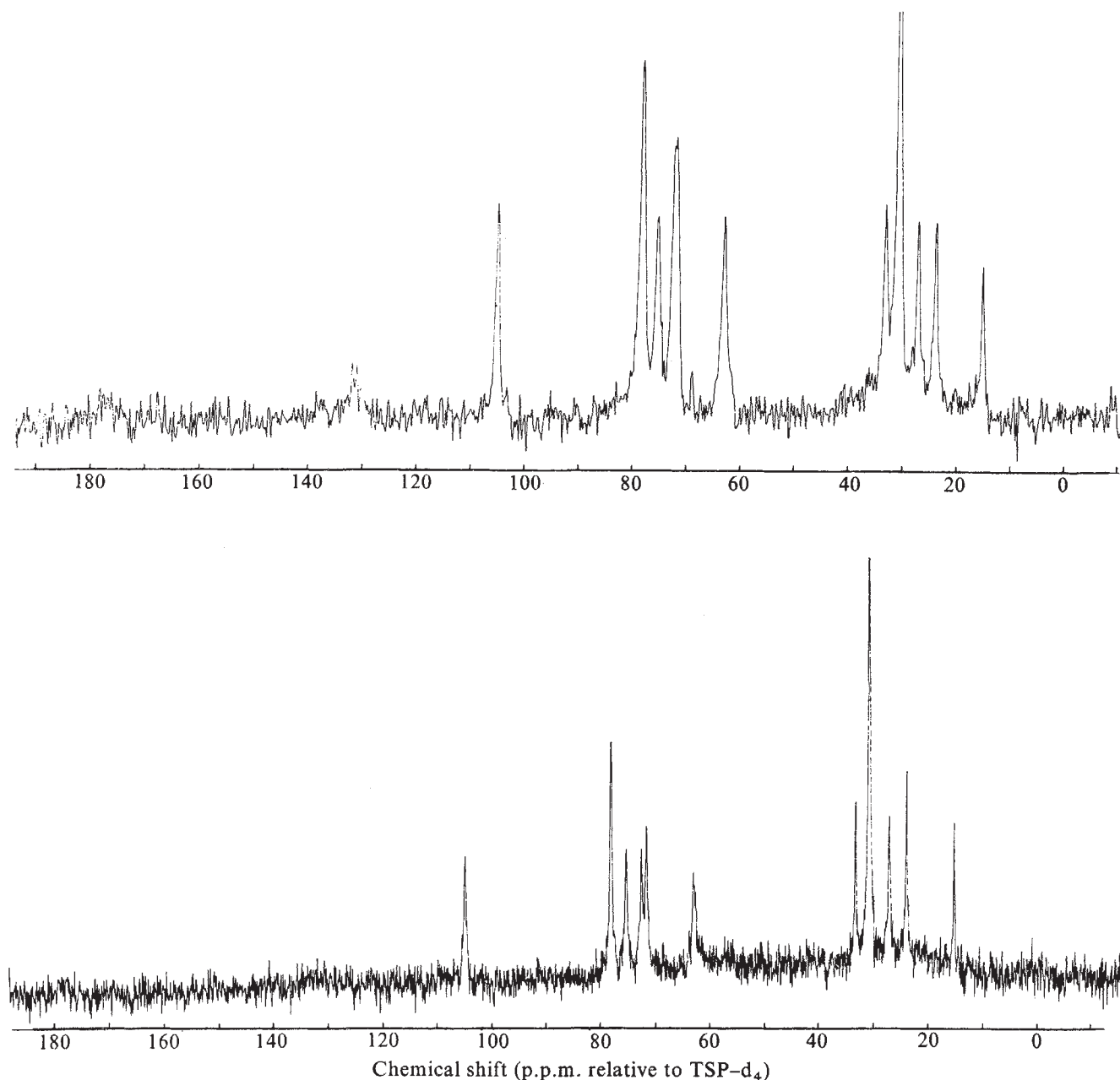


Fig. 1a, ¹³C NMR spectrum at 25.16 MHz of a solution of [14 (retinylidene)-¹³C] rhodopsin (0.5 mM) in 0.1 M KH₂PO₄ buffer (pH 7.0) with 100mM octyl glucoside. Acquisition time, 0.2 s; pulse delay, 0.2 s; 30° pulse angle; 12 mm tube; and 58,170 transients. An external lock was used; **b,** ¹³C NMR spectrum at 25.16 MHz of a solution of rhodopsin (0.5mM) in 100 mM octyl glucoside in 0.01 MKH₂PO₄ buffer with D₂O added for a lock (pH before D₂O addition, 7.0). Acquisition time, 0.8 s; pulse angle, 30°; 21 mm tube; 20,000 transients.

Previous work from our laboratory with model visual pigment chromophores, that is, 11-*cis*-retinal, N-11-*cis*-retinylidene-propylamine (NRPI), and N-11-*cis*-retinylidenepropyliminium ion (NRPI-H⁺), have shown that the chemical shift of the C14 position of the protonated Schiff base of retinal is at a significantly higher field (about 120 p.p.m.) than in free retinal and its unprotonated Schiff base (about 130 p.p.m.) (ref. 13 and J.W.S., G.D.M. and E.W.A., unpublished). Quite clearly, there is a large difference in chemical shift of the C14 position between that observed in ¹³C-enriched rhodopsin and the model protonated Schiff base of retinal. It is difficult to account for this large difference in terms of microenvironmental interactions. All interactions, steric, coulombic, ring currents, and so on, which are associated with protein folding would generally not lead to chemical shift differences larger than 2 p.p.m.¹⁴⁻¹⁷.

We have found that salts of model Schiff bases of all-*trans* retinal show the chemical shift of the C14 carbon in a pigment with a visible absorption maximum at 498 nm should be even further upfield than 120 p.p.m. (Fig. 3). In addition, there is a very small variation in the observed chemical shift for model pigments absorbing from 428 to 480 nm in cases where the counterion is associated with the nitrogen atom.

We have calculated the variation of the C14 chemical shift as a function of various counterion placements along the polyene chain from Pariser-Parr-Pople electron densities using the Karplus-Pople formula (ref. 18 and J.W.S. *et al.*, unpublished). These show no anomalous downfield shifts to 130 p.p.m. in a model pigment which has a significant bathochromic shift (about 140 nm) relative to the unprotonated Schiff base. Presumably this is due to the proximity of C14 to the protonation site; a significant negative charge is forced on C14 with protonation, and its magnitude varies little with counterion placement. Therefore, the chemical shift of C14 in a protonated Schiff base chromophore absorbing near 500 nm must be approximately 120 p.p.m. downfield from TMS. Attempting to position a charge such that a chemical shift of 130 p.p.m. would be observed should lead to a long wavelength absorption maximum near 350 nm.

The use of two anionic charges, one placed near the protonated nitrogen atom such as proposed by Honig *et al.*¹⁹ would also be expected to give shifts near 120 p.p.m. downfield from TMS for a pigment with a long wavelength absorption maximum near 500 nm since the C = N region in this case behaves similarly to the solution case. The above considerations clearly argue against the N-protonated retinylidene Schiff base model for the chromophore of rhodopsins.

It is most significant that the C14 shift for rhodopsin is consistent with the chromophore being an unprotonated Schiff

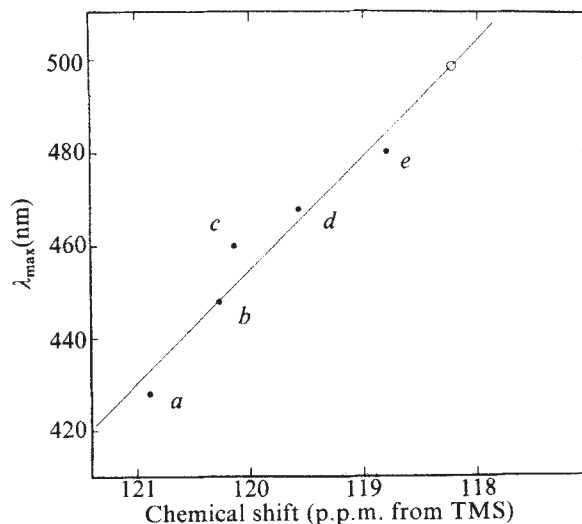


Fig. 3 Variation in shift of C14, δC_{14} , as a function of the long wavelength absorption maximum of all-*trans*-retinylidenepropyliminium ions, a, NRPI-HCl in acetone; b, NRPI-HCl in EtOH; c, NRPI-HCl in CDCl₃; d, NRPI-HBr in CDCl₃; e, NRPI plus a 100-fold excess of TEA in CH₂Cl₂. ○, Predicted value for a pigment with an all-*trans* chromophore with $\lambda_{max} = 498$ nm is 118.26 p.p.m.

base of retinal ($\delta C_{14} = 129.96$) or retinal itself ($\delta C_{14} = 129.59$ p.p.m.). Chemical evidence indicates that the retinylidene chain is bound to an ϵ -amino group of lysine on opsin rather than existing *in situ* as free retinal^{20, 21}. This would lend support to the original notion of Dartnall of an unprotonated Schiff base linkage²².

The above NMR results run counter to the conclusions drawn from resonance Raman spectra of rhodopsin. The band assigned to $\nu(C=N)$ of protonated Schiff base linkage by various investigators varies from 1645 to 1660 cm⁻¹ for rhodopsin having an absorption maximum at 498 nm¹³. Other peaks in the spectra—for example, $\nu(C=C)$ —vary to a much lesser degree (~ 4 cm⁻¹). Furthermore, bathorhodopsin and isorhodopsin both have a 1665 cm⁻¹ peak, whereas their visible absorption maxima are 543 and 483 nm, respectively; one would expect to find some variation of $\nu(C=N)$ with absorption maxima of the chromophore. This line of reasoning, together with the NMR results presented here, suggests that the peak in the 1650 cm⁻¹ region may be incorrectly assigned.

In conclusion, we point out that the NMR spectra of ¹³C-enriched rhodopsin leaves little question of the assignment of the probe signal while in resonance Raman spectra this is not the case. It seems evident from the results reported here that the Dartnall model of an unprotonated Schiff base chromophore in native visual pigments deserves further attention.

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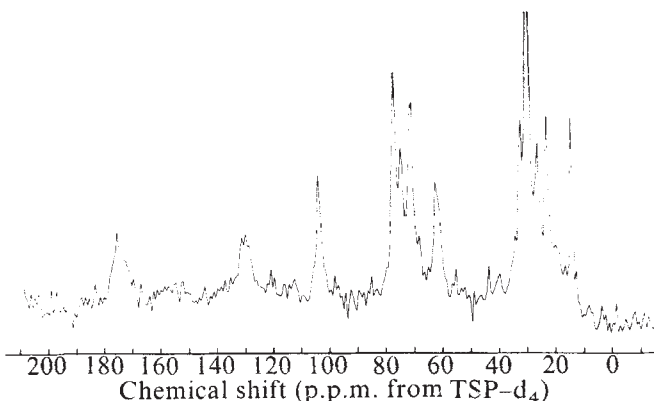
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Fig. 2 ¹³C NMR spectrum (14 KG field) of a solution of [14 (retinylidene-¹³C)] rhodopsin (1.0 mM) in 0.1 M KH₂PO₄ buffer (pH 7.0) with 100 mM octyl glucoside. Acquisition time, 0.2 s; pulse delay, 2.0 s; pulse angle, 90°; 8 mm tube; and 8092 transients. An external lock was used.



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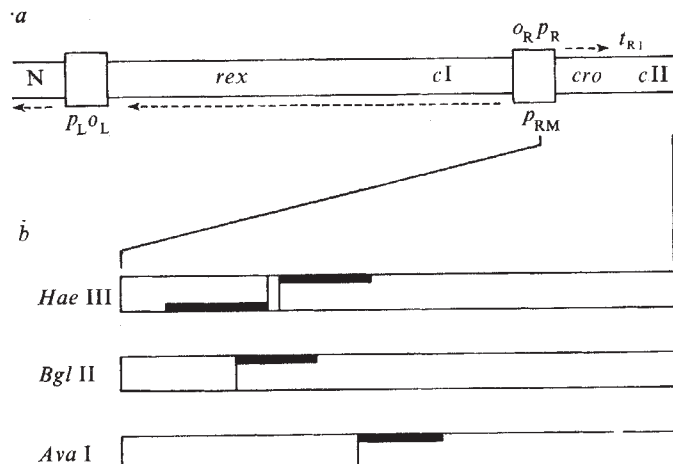
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Sequence of *cro* gene of bacteriophage lambda

KNOWLEDGE of the primary sequences of repressor proteins and the DNA operators with which they interact is essential for detailed study of the molecular aspects of the control of gene expression. The sequences of two repressors are known: the *cI* protein of lambda (ref. 1 and R. Sauer and R. Anderegg, personal communication) and the *i* gene product of the lactose operon of *Escherichia coli* (refs 2,3 and P. J. Farabaugh, personal communication). Here we report the DNA sequence for the structural gene of a third repressor, the Cro protein of lambda. This protein is of special interest both because of its small size (66 amino acids, as compared with the 236 amino acids of the *cI* protein and the 360 amino acids of the *lac i* gene), and because genetic evidence suggests that it interacts with the same operator regions as does the *cI* protein, although the

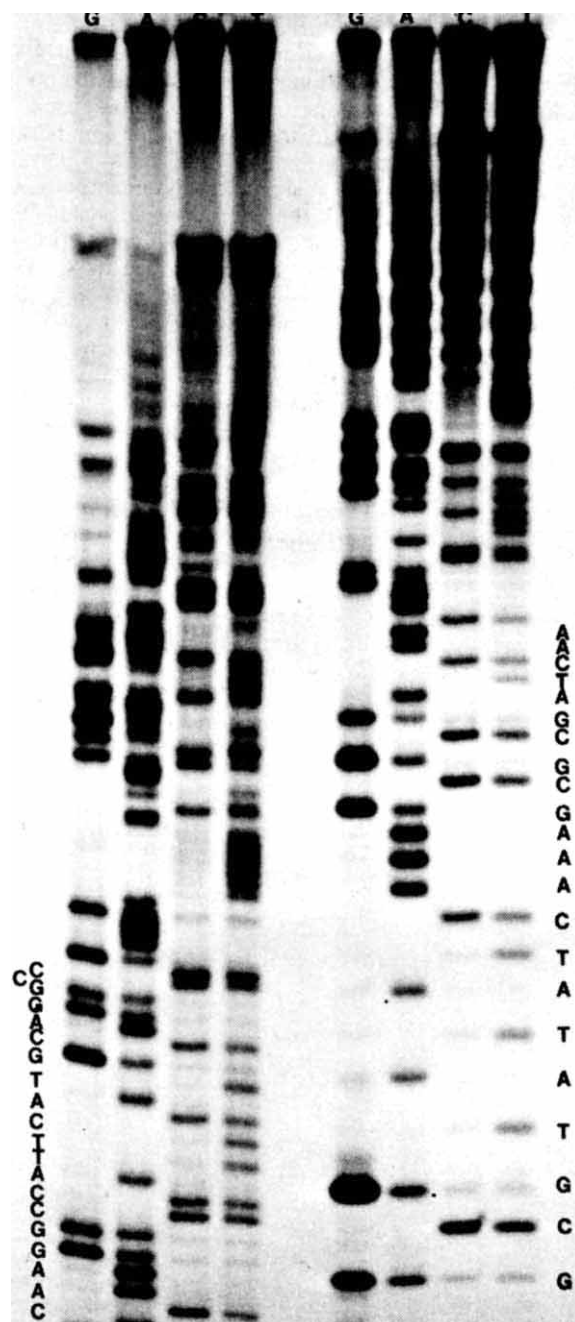
Fig. 1 *a*, Location of the *cro* gene within the immunity region of the lambda chromosome. *b*, An enlargement of the 550 base pair *Hin* II fragment which contains the *cro* gene showing the sites of restriction for three other endonucleases. The thickened lines indicate DNA strands which were end labelled and sequenced. The start point of the *cro* gene is 51 base pairs to the right of the left end of the *Hin* II fragment.



two proteins probably do not recognise exactly the same DNA bases⁴⁻⁷.

The location of the *cro* gene on the lambda chromosome is shown in Fig. 1a. DNA sequencing was carried out by direct chemical sequencing⁸ following the scheme of ³²P

Fig. 2 Autoradiogram of a polyacrylamide gel displaying 5' end-labelled oligonucleotide products resulting from partial, base-specific cleavage of the DNA fragment extending to the right of the *Bgl* II site shown in Fig. 1b. To sequence this region of the gene, DNA from a plasmid (A. Johnson, unpublished) containing the portion of the lambda chromosome extending from the rightmost *Hin* III site in the *cI* gene to the *Eco* RI site in the *o* gene was cut with *Bgl* II, dephosphorylated with bacterial alkaline phosphatase, and 5' end-labelled using [γ -³²P]ATP and T4 polynucleotide kinase. The resulting labelled fragments were digested with *Hin* II, and separated electrophoretically on a 3.5% polyacrylamide gel. The 440-base pair *Bgl* II-*Hin* II fragment containing the carboxy-portion of the *cro* gene was isolated from the gel and partially sequenced by the chemical procedures of Maxam and Gilbert⁸.



end labelling shown in Fig. 1b. Figure 2 shows an autoradiogram of a typical sequencing gel. The completed DNA sequence is shown in Fig. 3. Wherever possible the DNA sequence was checked by comparison with a catalogue of sequenced oligoribonucleotides generated by both ribonuclease T₁ and pancreatic RNase digestion of the RNA transcript of the region from *P_R* to *t_{R1}* synthesised *in vitro*. All oligoribonucleotides larger than tetramers have been uniquely identified. In this manner independent confirmation was obtained for over 70% of the sequence. The initial 27 bases of the sequence are in agreement with the sequence predicted for the 5' end of *cro* by Steege and Steitz⁹ from RNA sequencing of the message initiated at *P_R*.

Takeda *et al.*¹⁰ have isolated, identified, and determined the protein sequence of the lambda Cro protein. Their amino acid sequence is in exact agreement with that predicted by our DNA sequence. The occurrence of an ochre termination codon immediately beyond the COOH terminal alanine residue indicates that the cellular form of Cro is not processed. Comparison of the amino acid sequence determined for Cro with that of the lambda repressor indicates that no obvious similarities exist in the amino acid sequences of the two proteins.

Several aspects of the *cro* sequence are worth noting. (1) The 5' ATG of our sequence is three bases to the right of the putative ribosome binding site found by Steege and Steitz in the leader of the *cro* message⁹ and is 18 bases from the start point of transcription. (2) There is a region of perfect twofold symmetry in the DNA sequence involving bases 130–138 and 145–153. This hyphenated symmetry would give rise to a potential stem and loop structure in the RNA transcript of this region. The significance of this feature is not known; however, it does share the sequence ATAAA TTTAT with the right operator region *O_R*. (3) The region from base 106 to base 125 consists of a G-C rich region (106–113) followed at a short distance by the sequence TTTTTTAA (Fig. 2). This primary structure is reminiscent of known sites of *rho* independent transcription termination¹¹. But, *in vitro* transcription studies indicate that in the absence of additional factors RNA polymerase does not terminate transcription at this site (M.R., unpublished). (4) Finally, the distribution of bases utilised in the third or 'wobble' position of the codons of *cro* is approximately random (there is a slight bias against G's and toward A's and C's). There are, however, pronounced A-T or G-C rich regions within the *cro* sequence (for example, the region between positions 114 and 134 is

91% A-T; the region between 168 and 179 is 75% G-C; and the region between 180 and 201 is 77% A-T).

It should be noted that the region of the lambda genome which encodes the Cro protein apparently serves as template for leftward transcription (from the opposite strand) into the *cI* gene during establishment of repressor synthesis relatively early during phage development¹². Thus, the structural features noted above may reflect regulatory information involved in this transcription while concomitantly specifying the appropriate amino acid sequence for a functional Cro protein.

We thank M. Ptashne for helpful discussions, A. Johnson for making available plasmids containing *cro* gene and Alik Honigman for purified *AvaI* endonuclease. This work was supported in part by grants from the US NIH and NSF to M.P. T.M.R. is supported by an NIH postdoctoral fellowship.

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Fig. 3 DNA and protein sequences of the *cro* gene.

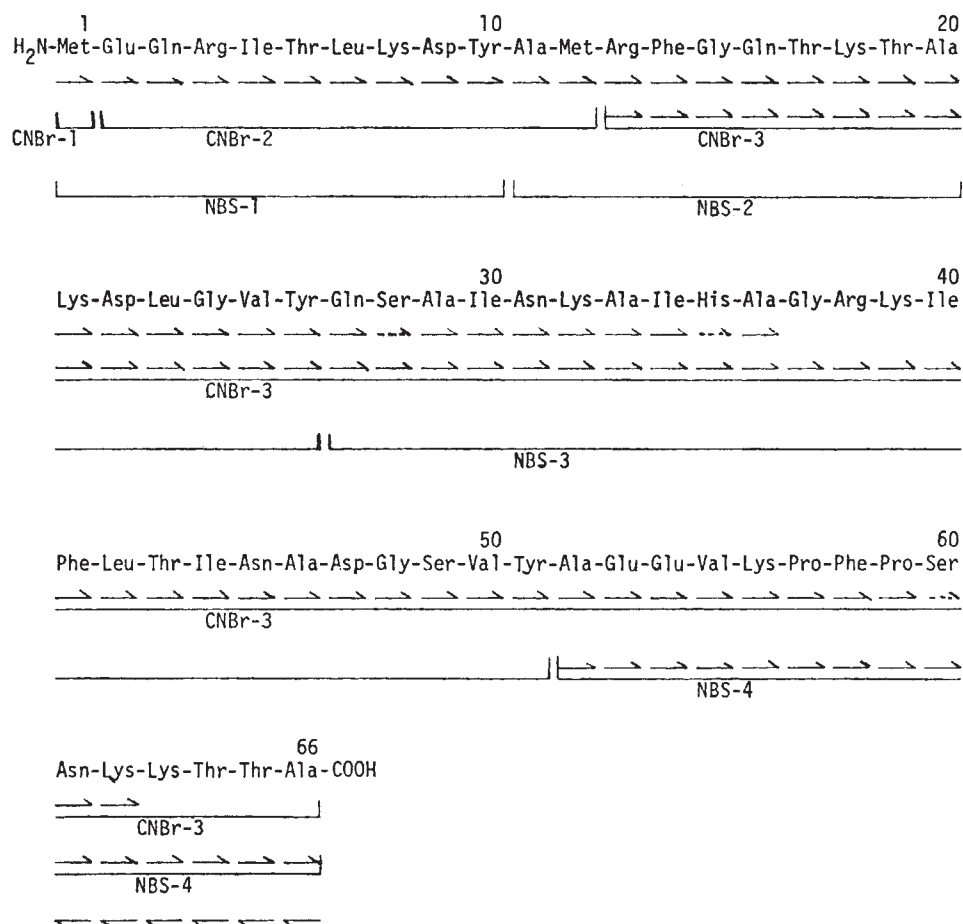


Amino acid sequence of Cro regulatory protein of bacteriophage lambda

THE Cro protein specified by bacteriophage lambda is a repressor of the genes expressed early in phage development; this temporal regulation is required for a normal late stage of lytic growth (see refs 1–5). Cro protein has been purified and shown to bind to the operator regions, *O_L* and *O_R*^{6,7}, used by the *cI* repressor protein to maintain lysogeny⁸; purified Cro also represses RNA synthesis initiated at the early promoter sites on λ DNA⁷. Among the features that make Cro interesting for further biochemical study are its timed physiological action^{1–5} and apparent role in regulation of DNA replication⁹. Cro is also convenient for structural analysis because of its extremely small size^{6,7}. Here we report the amino acid sequence of Cro protein.

The complete amino acid sequence of Cro protein has been determined by Edman degradation¹⁰ of the intact protein, and two series of peptide fragments, one derived from cyanogen bromide

Fig. 1 The amino acid sequence of Cro protein of bacteriophage lambda. Peptides are designated as CNBr when derived from cleavage by cyanogen bromide or as NBS when derived from *N*-bromosuccinimide cleavage; they are numbered sequentially in the order which they occur in the protein. Residue positions determined by automatic Edman degradation¹⁰ (→) or by hydrolysis with carboxy-peptidases²⁰ (←) are indicated with arrows. Amino acid sequence analysis was performed by using a Beckman automatic sequencer 890C and the Beckman DMAA peptide program. Identification of PTH-amino acids was by (1) gas-liquid chromatography¹⁷ on a Varian gas chromatograph 1840 A; (2) high-pressure liquid chromatography¹⁸ on a Waters Associates liquid chromatograph with μ Bondapak C₁₈ column; (3) amino acid analysis after HI hydrolysis¹⁹.



cleavage and the other from *N*-bromosuccinimide (NBS) cleavage. Digestion of the whole protein by carboxypeptidases A and B²⁰ further confirmed the sequence on the carboxyl end. The results are shown in Fig. 1 and Table 1.

The proposed structure contains 66 amino acid residues with a calculated molecular weight of 7,351 and has methionine and alanine as its amino and carboxyl termini respectively. The primary structure is in complete agreement with the amino acid

Table 1 Amino acid compositions and other properties of peptides of Cro protein

	CNBr-1	CNBr-2	CNBr-3	NBS-1	NBS-2	NBS-3	NBS-4
Lys	0.22	1.12(1)	7.00(7)	1.33(1)	2.12(2)	2.19(2)	3.11(3)
His			0.86(1)			0(1)**	
Arg		1.14(1)	2.09(2)	1.00(1)	1.10(1)	1.00(1)	0.10
Asp	0.38	1.23(1)	5.20(5)	1.39(1)	1.47(1)	3.08(3)	1.32(1)
Thr		0.83(1)	4.83(5)	0.91(1)	1.87(2)	1.30(1)	2.07(2)
Ser	0.61	0.12	2.84(3)		0.40	1.73(2)	1.06(1)
Glu		2.15(2)	4.69(4)	1.92(2)	1.47(1)	1.35(1)	2.26(2)
Pro			2.04(2)				2.00(2)
Gly	0.46	0.24	4.02(4)	0.65	2.16(2)	2.26(2)	0.41
Ala		1.31(1)	7.22(7)	0.35	2.40(2)	3.89(4)	2.34(2)
1/2 Cys							
Val			3.01(3)		0.93(1)	1.11(1)	0.96(1)
Met	1.0†(1)	0.87†(1)		0.30(1)	0.71(1)		
Ile		1.01(1)	3.84(4)	0.75(1)	0.56	3.80(4)	0.29
Leu		1.05(1)	2.19(2)	1.09(1)	1.16(1)	1.24(1)	0.10
Tyr		0.76(1)	1.84(2)	0(1)¶	0(1)¶	0(1)¶	
Phe			2.94(3)		0.71(1)	1.06(1)	0.80(1)
Total residue		11	54	10	16	25	15
Yield (%)	36.1	35.0¶	87.5	72.0	62.0	38.0	18.0
Purification steps*	A	A	A	A	AC	ABC	ABCD
Treatment of peptides†			F				F,G

*Purification steps, given in order of application. A, Gel filtration by Sephadex G-50 superfine; B, paper electrophoresis pH 6.4; C, paper electrophoresis pH 3.5; D, paper chromatography

†Treatment of peptide before sequencing. E, 4-sulphophenylisothiocyanate²¹; F, 2-amino-1,5 naphthalene disulphonic acid and *N*-ethyl, *N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride²²; G, acetylated cytochrome *c* (3 mg) was added in the sequencer cup to reduce the loss of peptide during automatic sequencing.

‡As homoserine

¶Low yield due to loss in handling

|| Detected by absorption of spirolactone

**Brominated

sequence predicted from the DNA sequence of the *cro* gene (ref. 11; and G. Hobom, personal communication). The composition of Cro protein based on the proposed sequence is Asp₃Asn₃-Thr₆Ser₃Gln₃Pro₂Gly₄Ala₈Val₃Met₂Ile₅Leu₃Tyr₃Phe₃Lys₈HisArg₃, which is 12 amino acid residues less than the composition previously reported⁷. This difference is largely due to renormalisation. Careful reanalysis of Cro protein of the highest purity and renormalisation (based on alanine) gives a composition that agrees with the one calculated from the sequence within experimental error.

The rules of Chou and Fasman^{12,13} have been applied to the amino acid sequence of Cro protein; these predict several alternating regions of α -helix and β -sheet, consistent with an overall globular conformation.

Although Cro and cI proteins recognise the same operator regions of λ DNA, there is no extensive sequence homology between the two proteins¹⁴. This indicates that the two repressors were derived by parallel evolution rather than by partial duplication of one of the two genes. The very different chemical nature of the two repressors probably reflects the different physiological needs of regulation during lytic development or lysogeny^{1,2,7}. Substantial biochemical differences have already been shown in the affinity of the two proteins for operator DNA^{7,8,15} and in the effect of operator region mutations on their binding properties⁶. There is also no detectable sequence homology between Cro protein and the eukaryotic chromosomal protein, histone H2B¹⁶, despite the close resemblance of their amino acid compositions.

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Active transport of picrate anion through organic liquid membrane

SEVERAL studies¹⁻³ have examined the concentrative transport⁴ of ions through bulk liquid membranes. The energy for the transport in these systems is the pH difference—the chemical potential difference of protons across the membrane. We describe here a redox reaction-driven transference system in which picrate anion, a lipophilic anion, moves not only against its concentration gradient but also against its electrochemical potential gradient through bulk organic solvent.

The apparatus is illustrated in Fig. 1. Two aqueous phases (L, left; R, right) are bridged by a dichloroethane layer (M) containing *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). The aqueous phase, L, contains an oxidising agent, ferricyanide, and R, a reducing agent, ascorbic acid. Figure 1 shows the typical data where the initial concentrations of picrate in L and R phases are the same (10^{-4} M). The M phase contains 10^{-4} M TMPD which is not equilibrated with picrate before the experiment. An increment of picrate in R phase signifies its corresponding decrease in the L phase. The concentration of picrate in R phase was found to increase and that in L phase to decrease linearly with time after a

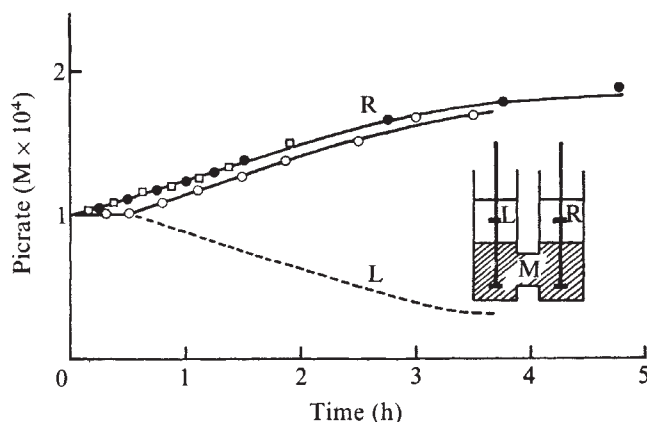


Fig. 1 Concentration of picrate as a function of time. Initial concentration of picrate was the same in L and R phases (10^{-4} M). The experimental chamber used was essentially the same as a U-shaped glass tube and is illustrated schematically in the inset. The volumes of the aqueous solutions designated as L and R are 15 ml and that of the organic bulk phase, M, is 40 ml. All three phases were agitated with a pair of glass stirrers at a speed of 180 r.p.m. The concentration of picrate was determined spectrophotometrically. The experiments were performed in a dimmed room to prevent light-induced degradation of TMPD. ○, Time course of changes in picrate concentration in R phase with the bulk phase not pre-equilibrated with picrate before the experiment. The aqueous phase on the L side contains 10 mM potassium ferricyanide and the R side, 10 mM sodium ascorbate; both solutions were buffered at pH 9.4 with 100 mM $\text{Na}_2\text{B}_4\text{O}_7\cdot\text{HCl}$. Because ferricyanide interfered with the spectrophotometrical determination of picrate, we determined the concentration of picrate in only R phase. The broken line is the calculated value of picrate concentration in L phase. ●, Data obtained with the organic phase equilibrated with picrate. The method of preparation of the membrane is as follows. A given amount of TMPD-2HCl was dissolved with minimal volume of distilled and deionised water (about 0.5 ml), which was poured into 40 ml of sodium picrate (10^{-4} M) buffered at pH 9.4 with 100 mM $\text{Na}_2\text{B}_4\text{O}_7\cdot\text{HCl}$. The free TMPD and the TMPD_{ox}^+ -picrate⁻ ion pair in the solution prepared as above were extracted into 40 ml of dichloroethane, which was washed again with picrate solution (10^{-4} M, pH 9.4). The organic solution was used as the barrier phase, M after the separation of two phases. Other experimental conditions were virtually unchanged. The rate of transference was independent of pH in the range pH 6.5 to 9.5. The pH was adjusted with phosphate or borate buffers (100 mM). □, Transport with voltage clamp at 0 mV. Other conditions as above.

short lag period, and finally a steady state was reached. Picrate was transferred from L to R phase against its concentration gradient.

The lag period became shorter or was absent when M phase had been equilibrated with picrate before the experiments. The slope of the linear portion was approximately equal to that obtained without prior equilibration. All experiments described below were performed using pre-equilibrated dichloroethane. The method of preparing the bulk phase (M) is described in Fig. 1 legend. Figure 2 shows the rates of picrate transport in conditions of varying concentrations of ferricyanide, ascorbate and TMPD. The rates increase with increase of the concentrations of these agents and reach approximately the same plateau level.

The membrane potential has been shown to be important in transport across membranes⁴⁻⁶. In the case shown in Fig. 1, the electromotive force (e.m.f.) was initially 160 mV, rapidly decreasing to reach a steady value of 120 mV with the polarity being positive in R with respect to L phase. The e.m.f. acts in the same direction as the picrate flux and hence it is possible that it might be responsible for part of the observed flux. To examine the role of the membrane potential, we clamped the potential difference between R and L phases at 0 mV by short-circuiting a pair of calomel electrodes inserted in L and R phases⁷. This made no difference to the observed results; that is, the membrane potential does not contribute to the transport of picrate in this system. This implies that picrate is being transported against its electrochemical potential gradient, that is, that the transport of picrate in this system is 'active'⁸.

Our results can be explained by the following mechanism. At the L interface, TMPD is oxidised to a cation^{9,10}, $\text{TMPD}_{\text{ox}}^+$ which serves as a carrier for picrate anion, picrate^- . The $\text{TMPD}_{\text{ox}}^+$ and picrate^- ion pair migrates down its concentration gradient within M phase. As the ion pair reaches R, picrate is extracted into aqueous phase by virtue of a discharge of $\text{TMPD}_{\text{ox}}^+$ due to the reducing agent. The neutral reduced form of TMPD_{red} diffuses back to the interface and the process is repeated. Structures of $\text{TMPD}_{\text{ox}}^+$ and TMPD_{red} are given in ref. 10. The importance of charging-discharging process of TMPD by the reduction-oxidation reaction is also suggested by the fact that in

acidic solutions, the rate of transference slowed down appreciably (data not shown). This may arise from protonation of reduced TMPD, $\text{TMPD}_{\text{red}} \text{H}^+$ possibly as a result of the lack of the charging-discharging process. The decrease in L phase and the increase in R phase of a negative charge resulting from the transfer of picrate^- from L to R are counterbalanced by the production of negative charge of $[\text{Fe}(\text{CN})_6]^{4-}$ from $[\text{Fe}(\text{CN})_6]^{3-}$ in L phase and by that of H^+ associated with the oxidation of ascorbate in R phase, respectively.

The transport of cations^{1,3} and amino acids² against their concentration gradient by coupling to a movement of the other ions in the opposite direction has been reported. It should be noted that no ion is co-transported or counter-transported in the system described here and that the energy for transferring picrate is supplied directly by the free energy associated with the chemical reaction. The substrate to be transported in this system is not restricted to picrate—any lipophilic anions capable of forming lipid soluble in pairs with $\text{TMPD}_{\text{ox}}^+$ may be transported. It may eventually be possible to prepare a membrane system in which a specific substance is transported by coupling of a chemical reaction.

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Erratum

In the article 'Mathematical models for the evolution of multi-gene families by unequal crossing over' by A. S. Perelson & George I. Bell, *Nature* **265**, 304-310 (1977), on p. 304, paragraph 1, line 10, 'randomly' should read 'tandemly'; p. 305, paragraph 6, lines 15 and 16 should be replaced by 'crossover there are three possibilities: (1) the number of copies of gene i remains the same, (2) increases by one, or (3) decreases by'; paragraph 6, lines 17 and 18 '[$1 - \lambda_n(t) - \mu_n(t)$], $\lambda_n(t)$ respectively' should read '[$1 - \lambda_n(t) - \mu_n(t)$], $\lambda_n(t)$ and $\mu_n(t)$, respectively'; p. 306, the right side of equation (3a) should read

$$P \frac{n_i}{N(t)};$$

p. 306, last line in left column, 'variance' should read 'second moment'; p. 306, the summation index should be ' i ' not ' I ' in the equation for $N(t)$ following equation (3b); p. 308, line 4, should read ' $P_{N_0 \rightarrow 1}(t)$ ' not ' $P_{N \rightarrow 1}(t)$ '; equations (12) and (13), ' P ' should read ' P_0 '; the left side of equation (15) should read

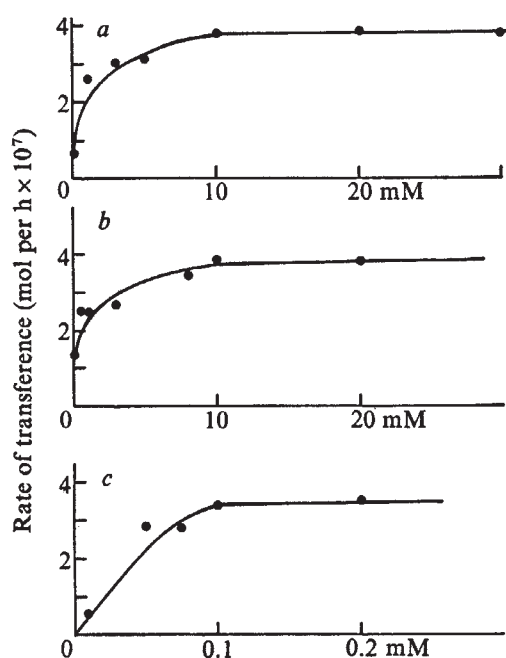
$$p_i(t) = \int_0^t \frac{dp_i(t')}{dt'} dt'$$

the left side of equation (16) should read

$$\int_0^t t' \frac{dp_i(t')}{dt'} dt'$$

paragraph 4, line 18, 'context³⁸' should read 'context³⁹'; p. 309, equation (18) ' $1 \leq \xi_1 \leq m$ ' should read ' $0 \leq \xi_1 N_0 \leq m$ '; equation (19) ' $0 \leq \xi_2 \leq m$ ' should read ' $0 \leq \xi_2 N_0 \leq m$ '; equation (20), the summation indices ' ξ_1 ' and ' ξ_2 ' should read ' $\xi_1 N_0$ ' and ' $\xi_2 N_0$ '.

Fig. 2 Rates of transport in conditions of varying concentrations of ascorbate, ferricyanide and TMPD. *a*, Rates plotted against the concentration of ascorbate where the concentrations of ferricyanide and TMPD are kept constant at 10 mM and 0.1 mM, respectively. *b*, Rates against $[\text{Fe}(\text{CN})_6]^{3-}$ with ascorbate and TMPD kept constant at 10 mM and 0.1 mM, respectively. *c*, Both ascorbate and $[\text{Fe}(\text{CN})_6]^{3-}$ are held constant at 10 mM. In all these experiments, the pH value is 9.4 buffered with 100 mM $\text{Na}_2\text{B}_4\text{O}_7\text{-HCl}$, and the initial concentrations of picrate is 10^{-4} M.



reviews

Immunological sourcebook

Elizabeth Simpson

Lymphocyte Differentiation, Recognition and Regulation. By David H. Katz. Pp. xiii+749. (Academic: London and New York, 1977.) \$42; £29.80.

THE scope of this book is no less than a review of the whole of current basic immunology. This is approached under a series of chapter headings dealing with the surface antigens of T and B cells and their respective ontogenies; receptors and specificity of these two major subclasses and their functional properties; regulatory interactions in immune responses, including genetic control and the role of the major histocompatibility complex; and finally, immunological tolerance.

This is a monumental assignment for one person to undertake. Two thousand three hundred references are quoted, most of them published since 1970. Dr Katz's approach has been to consider his selection of the relevant references under the headings he has chosen, in many cases quoting substantial details of the work under discussion. In chapters where he has integrated the results of several papers and the views of different authors, the story emerges as an interesting one, and his own views are illuminating. This successful approach is seen most in the chapters dealing with T-B cell cooperation, a field in which the author has been extensively engaged. One can argue with him as to his choice of relevant references, and the reservations, many times reiterated, with which he regards data gathered from *in vitro* systems, but the overall synthesis of these chapters is good; they are both informative and provocative.

The chapters dealing with subjects outside the author's own experimental field suffer from repetition and a lack of synthesis of the data and in the discussion presented. Some sections read like a catalogue of papers; for example, the section on H-2-restricted cytotoxic responses describes sequentially and in great detail each of the examples (this class of response to virus, hapten and minor transplantation antigens), and fails to underline the essential similarity of the systems and

the important biological implications of the findings. The general lack of cross-referencing, either in the text, or by way of the index (which is totally inadequate) is most noticeable and irritating in chapters which proceed by cataloguing rather than by synthesis. For example, the question of the biological importance of polymorphism in the major histocompatibility complex (MHC) comes up in the chapter on T and B lymphocyte surface antigens in the sub-section on biochemistry of MHC antigens (p78), in the chapter on "Functional Properties of T Lymphocytes" during consideration of H-2-restricted cytotoxic responses (pp 281-296) and in the chapter on genetic control of immune responses (p530 *et seq.*); and yet there is no cross-referencing of the discussions therein.

The organisation of the chapters under the chosen headings has led to some loss of clarity. This is particularly evident in the chapter entitled "Functional Properties of T Lymphocytes" subdivided into (A) "Regulatory T Lymphocytes" and (B) "Effector T lymphocytes". Such a classification, which quite arbitrarily considers helper and suppressor T lymphocytes under the first heading and mixed lymphocyte reaction proliferative T cells, cytotoxic T lymphocytes and T cells mediating delayed type hypersensitivity under the second, obscures the distinction between precursor T cells for any particular function and effector cells which have differentiated as the result of exposure to antigen. The distinctive phenotype of the precursor cells for each function has been documented from many laboratories using anti-Ly antiserum, and implies a precommitment to function before encounter with antigen. This data on precommitment is contained in several of the references quoted in the bibliography but Dr Katz makes only brief mention of the notion, quoting just one of the references in a manner suggesting that it contains the only data available on the point (p265).

In a different context Ly phenotyping is mentioned under the heading of "Surface Phenotype Antigens" (chapter VIII) and in the chapter on

"Surface Markers of T Lymphocytes" (chapter II). Discussion of the data, however, is scattered in the text, and these are not used to integrate earlier, inevitably less well defined observations on the separation of functional subsets of T cells. In some cases, as in the chapter on "Ontogeny of T Cells", they are not mentioned at all.

Chapters X, on "Regulatory Cellular Interactions", XII on "Genetic Control of Immune Responses" and XIII on "Mechanisms of Regulatory Cellular Interactions" obviously contain overlapping material. In some cases, the division chosen has fragmented and obscured the story; for example, the failure to follow through the discussion on T-B cooperation in chapter X with the data on factors, which is presented in chapter XIII. The factor chapter (XIII) itself is incomplete in as much that the genetically restricted factor of Erb and Feldmann is mentioned not here, but in the previous chapter, to which there is no cross-reference on this point, and the suppressor factor of Kontiainen and Feldmann is not mentioned at all.

There are some misquotations of references, some by omission (that is, failing to take account of the whole of the contents of a paper, in some cases, stating that the author(s) had not looked at a particular point, when they had done so) and some by addition (that is, fanciful addition to the data); but this must be almost inevitable when so many references are considered, and does not occur often enough to mar the value of the text and its very extensive bibliography for serious readers. Informed immunologists will be able to seek out the discussion and references they want by assiduous detective work which would not have been necessary had the index been adequate and the cross-referencing in the text more thorough. For less informed readers—for example, graduate students—this valuable sourcebook and bibliography will not be so accessible. □

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Nutrition and the brain

Nutrition and the Brain. Vol. 1: Determinants of the Availability of Nutrients to the Brain. Pp. xi+324. Vol. 2: Control of Feeding Behaviour and Biology of the Brain in Protein-Calorie Malnutrition. Edited by Richard J. Wurtman and Judith J. Wurtman. Pp. 313 (Raven: New York, 1977.) £19.50 each volume.

A NUMBER of years ago a distinguished neurochemist remarked that if people continued to take up neurochemistry at the rate obtaining at the time, then everyone in the world would be a neurochemist long before the end of the millennium. The rate at which books about nutrition and the brain have recently appeared seems to threaten a comparable natural disaster; and here are two more. The fact that your reviewer seems to be one of the few workers in the field who has not yet found the energy to produce one, may be the reason for a certain weariness, if not sourness in his approach and a developing propensity for denunciatory overstatement. But at least it will be agreed that a field with so much contemporary competition demands more than usual justification from the late arrivals.

Someone, somewhere, will find almost all of them very useful; but unfortunately the very misleading title of the series, *Nutrition and the Brain*, will not lead the literature surveyor to many of the better chapters unless they appear separately in the indexes under such headings as 'Cerebral Energy Metabolism', 'The Natural Diet of Primates', and so forth. However widely one interprets the word 'Nutrition', and however all-pervasively 'the Brain' controls the rest of physiology, the Editors have surely assumed too readily that their subject encompasses the whole of nutritional biochemistry and neurobiology.

Thus, if the young scientist entering the field happens to be fascinated by evolutionary aspects of the natural diet of Primates, then he will be as entranced as was your reviewer by the erudition of the first long chapter. But who is to blame him if he reflects that all the mammalian orders grow remarkably similar brains (in compositional and metabolic terms at least) whether they are gatherers or hunters, carnivores or vegetarians, ruminants or rodents; and that these matters are therefore unlikely to concern him. If he is a neurochemist, he will already have read one of Sokoloff's other excellent accounts of cerebral energy metabolism. If he is not, I suspect he

will not be much helped, even by such a classic account, until we reassure him that the subject is not one which bears at all directly on the problems of the starving billions or overstuffed millions of this wicked world.

The chapter on amino acid availability to the brain may at first sight seem more relevant, especially to those who have not yet caught up with the 'protein fiasco' and still believe that what the Third World needs most of all from us is steak. The whole topic of the blood-tissue relationships of the brain with regard to amino acids is a marvellous one for those interested in transport mechanisms or in certain in-born errors of metabolism. It is, however, of no direct interest in the present context.

It has been known for some time that certain putative neurotransmitters are synthesised within the brain from particular precursor amino acids; that the blood levels of these latter influence the brain levels of the former and are themselves influenced by dietary intake. It therefore persuasively follows that in this particular instance, something about the diet influences substances which are held to be to do with sleep, or mood, or otherwise with neurotransmission. This leads by delightful prestidigitation to the argument that since, under *in vivo* conditions, one metabolic pathway in the brain can be shown to be controlled by precursor substrate concentration; and since the latter is often derived from dietary sources, then who knows but that all the other metabolic pathways may not ultimately be under nutritional control as well? We can therefore discuss as many of them as we please. I suppose it depends what you mean by "ultimately". If this kind of argument persuades you of the relevance to '*Nutrition and the Brain*' of its energy metabolism; of amino acid, folic acid, B₁₂ and choline availability to the tissue, and of the role of B vitamins in nervous function, then these books are for you; but not, I fear, for me.

You may even be interested in the blood-brain barrier, other than as a convenient incantation for writing off phenomena which are otherwise difficult to "explain". In that case you can choose between chapter 2 in which the BBB is responsible for the brain's metabolic idiosyncracies but whose nature and location are uncertain; and chapter 3, in which the anatomical site is no mystery to the author and its properties quite well enough defined to "explain" a hopelessly mixed bag of transport mechanisms; and chapter IV which gives us yet another point of view on what may be the best-preserved neurobiological hoax of the twentieth century. At least the impartial reader

will not be lulled into complacency by any uniform consensus, and the hunt will continue for some kind of impermeable wash-leather separating the blood from the brain.

Volume 2 gets prematurely bogged down in a discussion of the control of eating behaviour in 145 pages with 827 references, as if the main elements of the main title (*Nutrition and the Brain*) have in some way become juxtaposed. The remaining three chapters, however, at last get down to the matter in hand. A chapter on the effects of undernutrition on brain morphology is mostly, and properly, about influences on brain development, although the authors complain that there is no information about the adult state in this respect. Could it not be that there is nothing interesting to see in the brains of starved adults (except in those exceptionally rare encephalopathies so beloved of the last chapters in tomes on clinical neurology)? I happen to think that in this whole, surprisingly lesion-free neuropathology of developmental undernutrition, the most rewarding contemporary growing-point may lie in current attempts at quantitative neurohistology. So, it is a bit disconcerting to be quoted here as not thinking much of the idea. There are some formidable technical obstacles to be overcome, however, before brain structures can be realistically counted, so that the whole of world knowledge in this area could be summarised on half a page with about four references; thus, it is perhaps to be expected that this chapter too dwells on matters outside its stated subject, or fills in the pages by reproducing chunks from elementary textbooks of neurohistology.

The second half of volume 2, however, blossoms forth with two quite outstanding chapters on the effects of widespread varieties of malnutrition on biochemical aspects of brain development, and on human behaviour. Both are masterpieces of clarity: the first in systematically describing what we know without ambiguity; the last in making it perfectly clear that there is no more difficult and confusing topic than the effects of infant malnutrition on human behaviour. The first, by Nowak and Munroe will be a classic reference point for a long time to come. The last, by Pollitt and Thomson should be a powerful antidote to those politicians and the less perceptive of their scientific advisers who still persist in that simplistic non-question, "Does infant malnutrition cause mental retardation?".

John Dobbing

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Low temperature physics

The Quest for Absolute Zero: The Meaning of Low Temperature Physics. Second edition with SI Units. By K. Mendelssohn. Pp. 281. (Taylor and Francis: London, 1977.) £5

Low temperature physics, for those who know little of it, often seems to represent the very epitome of scientific futility: a seemingly purposeless (and highly expensive) competition to see who can approach most closely the unattainable absolute zero of temperature. They should read this book. It would be very likely to change their minds since, as an apologia for one of the most fascinating and fruitful areas of physics to emerge this century, it could hardly be bettered.

Mendelssohn sets low temperature physics squarely in its historical and scientific context, close to the centre of the stage during that quite unprecedented explosion of knowledge which took place in physics in the early decades of the present century. Using an entirely non-mathematical approach, he explains with impressive clarity and accuracy how many of the fundamental concepts of modern physics arose from, or were illuminated and supported by, experiments on the novel states of matter which exist at very low temperatures.

The first four chapters are largely historical in structure, the story opening with Cailletet's liquefaction of oxygen (90K) in 1877, running on to describe the liquefaction of hydrogen (20K) by Dewar in 1898. This first section ends with an assessment of the achievements of Kamerlingh Onnes who, by liquefying helium (4K) in 1908 and discovering superconductivity, became the father of low temperature physics as we know it today. This section includes explanations of the isotherms of real (non-ideal) gases, critical points, cascade liquefiers and expansion engines.

Unusually, Mendelssohn considers how the personal attributes of the researchers may crucially have influenced the extent of their scientific achievements. For example, Dewar, portrayed as a brilliant but autocratic and quarrelsome loner, failed in his attempt to liquefy helium. The prize fell, instead, to the much more open, equable and diplomatic Onnes who possessed the inclination and ability to create a scientific laboratory in the modern sense, successfully coordinating the activities of a comparatively large complement of technical staff, colleagues, and visitors from other universities.

The next five chapters include vigorous and readily understandable accounts of topics such as the Third Law, heat, entropy, zero point energy, low temperature specific heats, quantisation, radiation, indeterminacy, gas degeneracy, quantum statistics of ideal gases, Fermi surfaces, Curie's Law, magnetic cooling and the various ramifications of superconductivity. Individual contributions to the developing conceptual structure made by such figures as Bohr, Born, de Broglie, Einstein, Fermi, Heisenberg, Nernst and Schroedinger, many of whom were known personally to the author, are carefully described and assessed.

At this point, the second edition diverges from the original, published in 1966. A whole chapter is now devoted to cryogenic technology, dealing with SQUIDS (superconducting quantum interference devices, used for measuring tiny magnetic fields), computer elements, superconducting motors and generators and, of course, superconducting magnets. The final chapter, on superfluidity, has also been extensively reworked to take account of recent advances, particularly in the new and rapidly growing field of superfluid helium-3. Even so, the pace of current cryogenic development seems

to have outstripped production of the book which, for instance, describes dilution refrigeration as capable of "temperatures approaching 0.01K in continuous operation", whereas 0.003K is now routinely being reached by this technique at Grenoble.

The coloured line drawings are helpful. There are also a number of photographs portraying most of the major scientific figures discussed in the text: of particular note is one entitled: "The heyday of low temperature physics. L. D. Landau in discussion with the author in Moscow, 1957."

As might be expected in a work with this breadth of vision, containing so much of the author's personal viewpoint of the scientific world, pun- gently and entertainingly expressed, there is much with which practitioners may take issue; but this in no way detracts from the success of the book as a whole. Like the previous edition, it can confidently be recommended to anyone who would like to know more about the behaviour of matter in that unimaginable abyss of coldness around and below 1K.

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Mammalian development

Developments in Mammals. Vol. 2. Edited by Martin H. Johnson. Pp. 241. (North-Holland: Amsterdam, New York and Oxford, 1977.) Dfl. 72; \$29.50.

THE second volume in this interesting and timely series continues in the style and format established in the first. There are both good and bad aspects to this. It was hoped that the small and inadequate typeface would have been improved but this volume is also difficult and tiring to read. Such is the speed with which these two volumes have been produced that the changes in typeface could not be introduced in volume 2. I for one look forward to their introduction in the next issue.

The good balanced mixture of developmental biology, reproductive physiology and biochemistry has been maintained. Two papers concern growth and maturation of oocytes, and transport and selection of sperm. These are followed by three papers each considering the cleavage stages of rodent development from slightly different viewpoints. This section is particularly welcome as the subject is somewhat controversial, with much circumstantial evidence concerning determination and differentiation of the two cell lines which emerge during cleavage. Here, in these three papers, all the

evidence is reviewed, and the various interpretations that have been put on the data are well discussed. Of the remaining four papers, one reviews the *in vitro* culture systems and their application to the peri-implantation period, whereas the other three address another hotly controversial subject—the involvement of steroid hormones in controlling implantation and in particular to what extent the mammalian blastocyst may produce steroids and thus perhaps control, at least in part, its own implantation.

Encouraged by editorial policy, authors have occasionally been speculative and have also used the book as an outlet for previously unpublished data. Although this policy has obvious advantages in publications such as this series, there are hazards since papers are edited rather than refereed, and somewhat one-sided arguments occasionally slip through the net. In this second volume, the editor has provided the best possible answer to this problem by gathering together several papers on one subject, thus presenting argument and counter-argument side by side. For scientists new to the field, this is especially useful. Although expensive, this volume is good value and augurs well for the next of the series.

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Shallow layers of the Continental Shelf

Exploring the Geology of Shelf Seas. By R. McQuillin and D. A. Ards. Pp. 234. (Graham and Trotman: London, 1977.) £10.50.

As with many books, the title is a little misleading. Although the subject matter is concerned with general techniques that may be used in mineral or even oil exploration, it would be incorrect to say that the book is concerned with exploration specifically. The NERC/IGS, of which the authors are members, uses the techniques described more as mapping devices than as exploration tools.

The purpose of the work is to describe how information about the shallow layers of the Continental Shelf down to about 300 metres or beneath Quaternary cover, may be obtained. Over the past decade, considerable attention has been given by the oil companies to the "deep geology" of the North-West European Continental Shelf resulting in the discovery of major oil and gas fields in almost every geological formation from the Carboniferous to the Tertiary. In contrast, however, very little is known of the near subsurface, and there are hardly any maps or data published on this topic, even by the IGS who have by their own confession done the most work. There is thus a large gap in our knowledge about the nature and extent of bottom sediments. This is the region with which the IGS is concerned and on which the book concentrates.

Seven of the chapters are devoted to geophysical techniques and only one to the acquisition of geological information by sampling and drilling. This tends to give the book a basic imbalance, and it is often not fully clear how the results of geophysical work are utilised by the geologists in making maps. Just such an account would have placed the book in perspective. Nonetheless, the book is clearly written and the reader should have little difficulty in understanding basic principles even if he is left a little in doubt as to the usage of the methods.

The opening chapter deals with the problems of position fixing at sea. This is a useful chapter by itself, as it is often difficult to find concise accounts explaining the various methods. Once at sea, two sorts of observation may be made. The first area is the sea itself, and the nature of the seafloor; the second, the strata beneath the sea bed, from which information may be

obtained either directly or indirectly. Ways of studying the former using echo-sounding and side-scanning solar techniques thus follow, but by far the greatest emphasis is placed not unnaturally on the latter: gravity and magnetics, but particularly continuous seismic profiling techniques, both sub-bottom—sparker—and deep penetration seismic. These tools, perhaps above any others, have provided the means of mapping large areas using relatively few geological control points.

Although well intended, the chapter on sampling methods does not fully relate to the rest of the book, and is itself somewhat disjointed, reading

Planetary atmospheres

The Physics of Atmospheres. By J. T. Houghton. Pp. xiii+203. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) £6.50.

PROFESSOR HOUGHTON has written a most interesting book with the intent "to introduce physics students at both undergraduate and graduate levels to the physical processes which govern the structure and circulation of a planetary atmosphere." He is completely successful. The book is very strong on the aspects of radiative transfer, as might be expected because Houghton's group is the world leader in this field. Chapter 2, "A Radiative Equilibrium Model", and chapter 4, "More Complex Radiation Transfer", are completely devoted to radiative transfer; the latter includes a most valuable discussion of how to proceed from molecular properties, such as line widths, to computations of radiative heating rates in realistic atmospheres. Chapter 5, "The Upper Atmosphere", also focuses on radiative transfer and contains a unique detailed treatment of the breakdown of thermodynamic equilibrium. Chapter 6, "Clouds", is mainly concerned with their radiative properties.

Chapter 7, "Dynamics", chapter 8, "Atmospheric Waves", and chapter 10, "The General Circulation", provide an excellent introduction to the role of the large scale motions in governing atmospheric structure. I found the approach here, and in chapter 9, "Turbulence", straightforward and efficient. It reminded me of some very clear lectures on the subject that I heard from Dr John Green of Imperial College in 1971. Houghton acknowledges a set of dynamics' lectures by Dr R. S. Harwood who was formerly associated with Dr Green. From these chapters, the student could easily tackle the more advanced texts on atmospheric dynamics.

Chapter 3, "Thermodynamics", in-

more like a catalogue than an explanation of how geophysical data is used to site sampling and drilling locations, or indeed of how maps are constructed.

One cannot hope, however, for everything, and the book will be of value in explaining little-known facets of geophysical techniques to students of the earth sciences who might otherwise find it difficult to locate the information.

John Brooks

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cludes a treatment of available potential energy as crystallised by Lorenz. This concept is carried, too, in later chapters and is most valuable in explaining structure. I have used it myself in lectures on the physics of the upper atmosphere. One can most easily perceive from this approach that the observed structure is the result of a continuous interplay between radiative and dynamical processes.

Chapter 11, "Numerical Modelling", and chapter 12, "Global Observation", again stress the radiative transfer aspects. Chapter 13 has the longest title of all, "Atmospheric Predictability and Climatic Change", but consists of only two pages of text and three figures, and seems to be an unnecessary afterthought which is not at the same level as the rest of the book. Chapter 6 is also a little too short to catch the essence of the physical aspects of clouds. These are, however, minor complaints, for the book as a whole is very well written, and does not pretend to provide all of the physics of atmospheres.

I learned much from working through some of the examples (provided with solutions) which are an integral part of the text. The interested student could, in fact, teach the subject to himself with the text, the useful information in the appendices and a hand-held calculator. Although references are provided, little from them is needed to work through the text. I recommend the book to all physics students interested in atmospheres. To all physics students, the text presents live examples of the laws of physics, which may replace the older examples of steam engines and ice skaters. Many first-year meteorology students would also benefit from working through this text. An early overview of the subject might be very helpful before immersion in two years of concentrated courses at the graduate level.

Reginald E. Newell

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Quirks and strong points

Psychologists on Psychology. By David Cohen. Pp.360. (Routledge and Kegan Paul: London and Henley, 1977.) £6.95.

PSYCHOLOGY nowadays is a very mixed bag, and indeed some would query whether it is really just one bag. David Cohen's book, which is a series of interviews with successful psychologists, illustrates very well how far apart from one another they are. It is not so much that they disagree, though disagreements there are in plenty in this enterprising book. What is much more striking is that the different psychologists featured in this book are often so far apart in their subject matter, their approach, their conceptual frameworks, and their aims, that even mutual criticism is out of the question.

The interviews which David Cohen

gave to these stars of the behavioural world seem to have been well planned, and the questions are searching enough to bring out the subject's quirks as well as their strong points. Perhaps Cohen should have been more critical but even here there is an advantage of some interest, because the calmness of the interviews seems to have brought out into the open a staggering complacency in some of the psychologists concerned.

David Cohen's attempts at the beginning and end of the book to find some link between the backgrounds and personalities of the different interviewees—a psychology of psychology—are almost certainly ingenuous; these people are as diverse as their work. The interviews which form the core of this book, however, give a clear, conveniently quick though often disquieting picture of what many of the present leaders of psychology think they are up to.

P. E. Bryant

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Androgen action

The Mechanism of Action of Androgens. By W. I. P. Mainwaring. Pp. x+178. (Springer: New York and Berlin, 1977.) DM65.40; \$28.80.

THIS is a useful monograph written by an acknowledged expert in the field of androgen action. The book is an attempt to describe the molecular events associated with the entry of testosterone into androgen-sensitive cells and the subsequent metabolic changes associated with the presence of the hormone in these cells.

The author uses a temporal approach to the problem of the analysis of androgen action and, as usual, much of the material is drawn from experiments on the rat ventral prostate. The author is careful to point out the reservations which must be applied in generalising from this animal model to any other species or tissue.

The core of the book is devoted to an account of the initial events and the early and late events associated with androgen stimulation of target tissues. The author points out that, although androgens influence transcription and the synthesis of RNA, they may also affect other processes. The manner in which androgen metabolism in the target tissues influences the mode of action of androgens is considered in detail. The high affinity binding sites,

the intracellular transformation and the subsequent nuclear retention of the hormone are reviewed. The need to isolate and characterise the androgen receptor proteins is stressed. It is a pity that the author did not devote more attention to the effects of androgens on muscle and perhaps develop a working hypothesis or speculate on how they may affect this large body mass.

In the latter part of the book, the author critically reviews his own studies showing that some of the actions of androgens are explicable on the basis of the increased production of selected mRNAs and ribosomes. Thus, androgen action is at least in part an enhancement of genetic transcription.

The author writes: "The halcyon days of studies on the mechanism of action of androgen are drawing to a close." In fact, this excellent text is a clear indication that this is not so. Future research on the mode of action of androgens may, however, require more sophisticated approaches and more elegant tools. The book is clearly written and the reader gets the impression of being led through some of the mysteries of androgen action as in a series of lectures. The text has the stamp of authority. It is written by a scientist who has in fact uncovered many of the interesting facts on the mode of action of androgens. The book will be valuable to researchers and to university teachers concerned with the mode of action of steroid hormones in general and androgens in particular.

G. S. Boyd

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Geomorphology and remote sensing

Remote Sensing in Geomorphology. By H. Th. Verstappen. Pp. x+214. (Elsevier Scientific: Amsterdam, New York and Oxford, 1977.) Dfl.98; \$39.95.

THIS well produced book will be valuable to the postgraduate or advanced research worker, rather than as an undergraduate textbook. A large amount of material is covered in a somewhat condensed style. Perhaps one of the chief merits of the book lies in its extensive bibliography of sources in English, German, Dutch, Russian and French languages. Of the 214 pages, approximately eleven are close printed lists of references, although some sources seem to have too brief a description, which may not be sufficient for procurement.

The pages are a source of constant stimulus: a technique or idea is introduced, and briefly described, but the reader will have to go to the original source for full details. This is perhaps a natural result of a text from an authority with the length and breadth of experience of the author.

The illustrations are plentiful and well produced. Some are in colour, and a notable feature is the number of stereopairs printed side by side for viewing with a pocket stereoscope.

Subjects covered include an historical introduction, a description of the types of imagery available, and available methods of interpretation. Chapters are devoted to image analysis based on relief (stereoscopy) and density criteria; pattern and texture are subsumed within these headings.

Geomorphology itself is divided into genetic and environment divisions, the latter including treatment of land systems methods of survey. This means that environments and major zones such as coastal, glacial, tropical and fluvial are discussed using examples hard upon one another's heels, with photography, radar, thermography and satellite imagery also intermingled. The examples used include lunar and planetary geomorphology. A final chapter deals briefly with such vital items as relative costs and fieldwork methods.

This is a most stimulating book, but one to be dipped into and used for reference or browsing. Because of the condensed staccato style, however, it is somewhat indigestible.

J. R. Hardy

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● In the review of *Perpetual Motion: The History of an Obsession* (Nature, 20 October, 269, 731; 1977), the price should have read £5.50.

Biochemistry and pharmacology of venoms

Venoms: Chemistry and Molecular Biology. By Anthony T. Tu. Pp. 560. (Wiley-Interscience: London and New York, 1977.) \$43; £25.90.

VENOMS have always had a peculiar fascination. How do they kill animals (and, in particular, humans)? What do they contain, and can any of the components be put to use either as tools in research or as therapeutic agents? Before the advent of modern chromatographic techniques, not much progress could be made in the field but since then there has been (to say the least of it) no shortage of papers. The quality of some of these has, however, been exceedingly bad. Separation procedures still require some skill and if the skill is lacking they lead to unspecified mixtures. The desire to inject such mixtures (or, indeed, whole venoms) into animals and see what happens is (apparently) strong. It ought always to be firmly resisted.

All of this means that to write a book on venoms is a formidable undertaking. It needs a considerable knowledge of the relevant aspects of chemistry, biochemistry and pharmacology and, if one is to deal with the effects of venoms in humans, of clinical medicine as well. Enough to daunt the strongest spirit. Professor Tu admits this in his preface and uses the phrase "monumental task". He has, in the event, produced a book containing over four hundred pages devoted to snake venoms (his own research interest) and rather less than one hundred to other animal venoms (scorpions, bees, wasps, and so on). One could hold the view that this is a trifle unbalanced, since (for example) the components of bee venom have been extensively investigated both chemically and pharmacologically. On the other hand, it is true that a bewildering variety of peptides and proteins have been found in snake venoms and some of these have proved to be extremely useful. For example, the phosphodiesterase contained in many snake venoms is an invaluable tool in the determination of nucleotide sequences in nucleic acids. On these grounds, the long section of the book on the enzymes present in snake venoms can, no doubt, be justified.

I must confess, however, that I found the book to be essentially uncritical. Most of the papers quoted seem to be given equal weight when clearly some of them (all too many, I fear) are un-

satisfactory on chemical or biological grounds or both. What, for example, is the point in giving a list (p509) of the values reported for the (so-called) LD₅₀ of apamine in mice when it is perfectly clear that the correct value is that given by Habermann (in 1972, incidentally, and not as quoted) and subsequently confirmed by other workers? How does one feel about the (supposed) component of bee venom called "cardiopep"? Ought one not to entertain the suspicion that the biological effects ascribed to it are really due to noradrenaline (shown to be present in the venom, as distinct from the venom gland, by Banks *et al.*, 1976, and not, as given, by Owen, 1971)? Again, what about minimine? This is certainly my favourite substance (or non-substance) because its biological activity is supposed to be the produc-

tion of miniature flies from the larvae of *Drosophila melanogaster* (whoever would think of such an experiment?) One could continue, but the list of doubtful substances and even more doubtful biological activities would be long and tedious. In defence of Professor Tu, I must say that, in my view, no one man could read and assess critically all the literature in this area.

It is a pleasure to be able to say that the book is well produced, well laid out, and that the references are remarkably up-to-date and (for the most part) remarkably complete. The book will certainly be of value (if only for the references) to workers in the field and to those wishing to enter it.

C. A. Vernon

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Basic statistical handbook

A Handbook of Numerical and Statistical Techniques. By J. H. Pollard. Pp.xvi+349. (Cambridge University: Cambridge, London and New York; 1977.) £13.50.

WHEN books on basic statistics continue to appear with amazing regularity, it is refreshing to find one which more than permutes the 'usual material'. This book not only describes essential statistical techniques but gives a welcome priority to elementary numerical methods.

The author aims to help computer users, firstly to understand the output of statistical packages, and secondly to adapt these and similar packages for programmable calculators to suit their own requirements. Although it is not a textbook of elementary numerical and statistical techniques, its uniformity and clarity of presentation, together with its comprehensiveness, should give it wide appeal. A basic knowledge of mathematics is assumed.

The contents have been skilfully divided into three parts. Part I (seven chapters), after a brief introduction to essential basic mathematics and a helpful discussion of sources of error, describes numerical methods for locating the roots of non-linear equations, data smoothing, integration, differentiation and interpolation. It finishes with a variety of useful techniques including the regrouping of grouped data, the fusing of smooth curves and steepest descent.

Basic statistical techniques, except regression, are presented in part II (seven chapters) which, after briefly

introducing probability theory, describes the important frequency distributions including the Pearson system. Two chapters devoted to hypothesis testing and point and interval estimation provide a broad survey of parametric and distribution-free statistical tests. Each test is presented in a standard format which facilitates both comprehension and correct application. This part ends with three special topics—random numbers, data transformation, and censored and truncated distributions.

Part III (four chapters), devoted to the method of least squares, describes simple linear regression (with and without matrix notation) and most of the associated statistical tests. (Unfortunately, the statistical comparison of two or more regression lines has been omitted.) Curvilinear and multiple linear regression are presented succinctly, using matrix notation, and this part ends with an introduction to non-linear regression.

Throughout the book, each technique is separately referenced and well illustrated with a fully-worked example, usually from the life sciences. Specialised tables are sensibly interspersed with the text, while those of more general application are placed in an appendix. Every chapter includes a selection of exercises though unfortunately the answers are not given.

This handbook has been carefully assembled, it is easy to use, and cross-referencing is kept to a minimum. It should be a valuable asset to the scientist and the statistician.

A. L. Johnson

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obituary

Bruce C. Heezen

PROFESSOR Bruce C. Heezen of the Lamont-Doherty Geological Observatory, New York, died on 21 June 1977 at the age of 53 of a heart attack while working on board the U.S. nuclear research submarine NR-1 studying the Reykjanes Ridge south of Iceland.

With his late colleague, Professor Maurice Ewing who was founder and director of the Lamont (later Lamont-Doherty) Geological Observatory, Bruce Heezen played a major part in the rapid post-war expansion of our understanding of the morphology and geology of the deep ocean floor, on which the now widely accepted theory of plate tectonics is based.

During the war the techniques for studying the deep ocean improved immensely so that in the early 1950s all sorts of new geological and geophysical data were being collected from the increasing number of research cruises. With Maurice Ewing, Bruce Heezen saw the enormous potential in the thorough compilation and analysis of these data, especially the morphology, on a world-wide scale. Because of the restrictive attitudes of the U.S. Navy on the publication of contour charts, he was forced to develop, with the cartographic skills of Marie Tharp, the physiographic diagram style of presentation of seabed morphology. These charts, several of which were published by the National Geographic Magazine, have been widely used by those interested in the oceans and have contributed significantly to an appreciation of what lies beneath the sea surface. His analysis of ocean floor morphology, initially of the North Atlantic and published as a Special Paper of the Geological Society of America in 1959 but later extended world-wide, became the basis of a physiographic classification used now by oceanographers, geologists and even by lawyers.

During these morphological studies in the late 1950s he made what I believe to be his greatest contribution to the earth sciences. He recognised that the major ridge systems which lay roughly in the centre of many oceans were in fact linked into one continuous, although sinuous, mountain range 40,000 miles long and covering an area equal to that of all the continents combined. He found that the axis of this mid-ocean ridge system was associated with shallow seismicity and was able to predict the existence of hitherto unknown ridges southeast

of Africa and across the Arctic ocean. Along the axis Heezen, and his colleagues Tharp and Ewing, noted a more or less continuous valley which was morphologically similar to and actually linked to the Red Sea and the East African rifts, and also to the tension cracks in the central Icelandic graben. It was clear that along this 40,000 mile axis the crust of the earth was under tension and splitting apart. Heezen saw this initially as evidence for an expanding earth but dropped this idea when the subduction mechanism was discovered to absorb the excess crust and global plate tectonic theory developed.

Although Heezen was able to view the oceans on a global scale, he was concerned with all scales of deep-sea geological processes. He recognised that far from being quiet passive regions, the abyssal deeps were disturbed by fierce and destructive turbidity currents which contributed to the cutting of submarine canyons, transported sediment great distances along the sea floor and which gave rise to abyssal plains. He was interested in all modes of sedimentation and pioneered the study of deep-sea bedforms, such as sand waves and dunes, and their relation to near-bottom ocean water movement. In his search for details of bottom processes he exploited the use of bottom photography to the full, superbly and readably presented to the general public in his book with Hollister, *The Face of the Deep* (1971) nominated for a U.S. National Book Award, and in the last decade he made numerous dives in research submersibles to observe the bottom directly.

Heezen was much concerned with the practical problems presented by the deep ocean. For years he advised the cable industry on the potential hazards to deep-sea cables from geological causes and much of his submersible work was directly related to naval requirements. He was an ardent supporter of international collaboration, especially the GEBCO bathymetric chart project, and pioneered the cartographic presentation of geological and tectonic data for the Commission for the Geological Map of the World.

Born in Iowa, Bruce Heezen obtained his bachelor's degree from the University of Iowa in 1948 and his Ph.D. from Columbia University in 1957. He joined Maurice Ewing in the formative stages of the Lamont Geological Observatory and spent his work-

ing life there, becoming assistant professor in 1960 and associate professor in 1964. He was an enthusiastic and hard-driving seagoing scientist dedicated to using ship-time to its fullest extent. In the same way he used his time ashore to the full, often hard at work until the small hours in his rambling and paper-filled house on the Hudson River and yet never too busy to devote time to his students. Neither did he stint himself in some of the pleasures of life. He was always good for a party, beaming, boyish in appearance and heavy in build. He had a strong and provocative personality, not mincing his words when he felt strongly about an issue, and it was tragic, but perhaps inevitable, that his early collaboration with the equally strong personality of Maurice Ewing should have changed into a bitter quarrel in which both parties as well as the science suffered.

Heezen's contributions to science have been recognised by the award of the Cullum Geographical Medal and the Francis Shepard Medal for excellence in marine geology, and in June this year by the award by the American Geophysical Union of the prestigious Walter H. Bucher Medal for his life's work of "original contributions to the basic knowledge of the earth's crust."

His loss will be keenly felt by his life-long collaborator, Marie Tharp, his many colleagues, friends and students, and by the whole marine geological community who owe so much to his energetic research, his insight into the deep oceans of the world and his inspiration of his students.

A. S. Laughton

Howard Hinton

PROFESSOR Howard Everest Hinton FRS died on 2 August 1977, aged 64. He was one of the few remaining polymaths in the field of biology, having wide interests in many branches of the subject. However, he had a remarkable ability to become expert in any topic within a remarkably short time, so that few of his acquaintances, meeting him in connection with one facet of his interests, realised that this was not his major subject of study. Even his friends were often surprised by discovering his involvement in new fields of interest in science and the arts, as well as by his political naivety.

Hinton was born in Mexico, and his

reminiscences of his childhood suggested that his early education was far from conventional. It clearly did not allow him to achieve his academic potential, for he was not thought well grounded enough to enter the University of California at Berkeley, and had to content himself with admission to St José State College. However he so distinguished himself that he was soon able to transfer to the Berkeley campus. As a student he is said to have been aggressive and somewhat anti-social, with political attitudes and a concern for the underdog which, even at Berkeley, were forty years ahead of their time. Nevertheless he was clearly a brilliant student, and after taking his B.Sc. he moved to Cambridge, England to take his Ph.D. under the supervision of Dr A. D. Imms.

His main academic preoccupation at that time was beetle taxonomy, but his wider interests were already apparent. He went on scientific expeditions to his birthplace, Mexico, in 1933 and 1934, and in 1937 to Peru, Bolivia and Brazil. In 1939 he was appointed Assistant Keeper at the British Museum (Natural History) in London, first to work on what was to him the new subject of orthoptera (he soon made himself an expert) and then on the group which were still his main interest, beetles. He caused some concern to the authorities at the museum, for he was not prepared to adopt the gentlemanly working hours (10 to 5) then generally accepted. He slept—or worked—night after night in the museum during the blitz and often even when there were too few bombs falling to make this respectable. His scientific output was prodigious, including a 350 page monograph on the beetles affecting stored products in addition to a great many solid papers on insect taxonomy and phylogeny.

His work at that time included studies of larval lepidoptera, which led to a general interest in lepidopteran phylogeny. Some of his colleagues at the museum thought that he was devoting too much time to such studies and not to conventional taxonomy. Hinton was therefore glad to move, in 1949, to Bristol University as Reader in Zoology. This gave him greater freedom to choose his fields of study, a choice of which he made good use. He was elected FRS in 1961, to a personal Chair of Entomology in 1964, and became Professor of Zoology and head of the Department in 1970. At Bristol his best known research was his delicate work with the scanning electron microscope, with which he studied and elucidated the functional morphology of many forms of insects and their developmental processes.

Surprisingly for someone not brought up in the English countryside, Howard Hinton was an excellent field naturalist familiar with the flora and fauna of Britain. In fact he said that he derived his inspiration for his apparently esoteric laboratory work from his observations of living creatures in their natural environment. That his interests were not restricted to insects was further demonstrated by his production (with A. M. S. Dunn) of an attractive book on Mongooses.

As well as being an acute observer, a formidable synthesiser and a stimulating teacher, Hinton was an efficient and hardworking editor. He started and brought to success two major scientific periodicals, the *Journal of Insect Physiology* and the *Journal of Insect Biochemistry*. Most scientists would have felt that editing only one such publication was, in itself, a full-time job, yet Hinton managed both without any apparent reduction in his research or other activities. From 1969–70 he was also an effective President of the Royal Entomological Society, and in 1972 of the British Entomological and Natural History Society. Even towards the end of his life, when he knew that he was unlikely to survive until the date of his projected retirement in September 1978, he continued his activities with undiminished vigour and even completed his massive, three volume, monograph on insect eggs. His work in many fields will be appreciated for many years to come.

Kenneth Mellanby

Leonard Eastham

PROFESSOR L. E. S. Eastham died on 19 July 1977 at the age of 84. Many former students remember him with gratitude and affection, for his understanding of their problems and his effective and unspectacular help. He came originally from Lancashire, and studied agriculture at the Harris Institute, Preston. This course was interrupted by the 1914–18 war, when he served in the Special Brigade of the Royal Engineers. After demobilisation he crossed the Pennines to Leeds, and studied zoology under Professor Walter Garstang. From 1921–27 he was lecturer in zoology at Birmingham University.

In 1927 he was appointed Lecturer in Advanced and Economic Entomology at Cambridge University. At that time Cambridge gave scant recognition to those coming from universities other than Oxford and Trinity College, Dublin (holders of provincial doctorates

were officially 'Mister') and Eastham's position was, at first, not a comfortable or an easy one. I remember him saying that some of his colleagues were so anxious to show their high social status that they forgot to behave like gentlemen. However, he was soon warmly accepted by his students, particularly those taking entomology in Part 2 of the tripos. He proved an inspiring teacher; his knowledge of agriculture, in a department where few had such practical experience, made the subject live, and a number of his students went on to important work in economic entomology in many lands.

From 1932 to 1958 Eastham was Professor of Zoology at Sheffield. Before the 1939–45 war the department was tiny, with only two other academic colleagues. Except for large classes of medical and dental students (1st M.B.) the number of undergraduates was not great, but there were separate classes for intermediate, general degree and special honours students, and a heavy teaching load which would overwhelm most present-day academics. Eastham bore his full share of the teaching, always maintaining his standards. He ran the department efficiently with next to no secretarial help. And he continued actively in research, producing a stream of scholarly and original papers.

After the war, the university, and the zoology department, grew rapidly to many times its previous size. Eastham was Dean of Science, and, from 1946–50, the first Pro-Vice-Chancellor. These and other outside duties did not prevent him from continuing his close and valued contacts with his junior colleagues and his present and past students.

From 1946 until his retirement Eastham played an important part in the creation and development of the new universities being established throughout the British Empire. In 1946 he was a member of a party under Sir William Hamilton Fyfe's chairmanship which visited West Africa and selected the site for the future university of Ibadan. He was a member of the Inter University Council for Higher Education in the Colonies (now for 'Overseas') and its executive, and he paid many visits to the embryonic universities and served on the Council at Ibadan. His visits were always appreciated; unlike many of the 'experts' who were similarly involved he always took the trouble to learn the facts, especially when political and other troubles arose, and his shrewd but sympathetic assessment helped and encouraged those trying to establish institutions of high academic standards under difficult conditions.

Kenneth Mellanby